Eesti Maaülikool
Estonian University of Life Sciences

UTILIZING TRITROPHIC INTERACTIONS TO DEVELOP SUSTAINABLE PLANT PROTECTION STRATEGIES FOR OILSEED RAPE

TRITROOFILISTE SUHETE RAKENDAMINE JÄTKUSUUTLIKU TAIMEKAITSE STRATEEGIA LEIDMISEKS RAPSILE

RIINA KAASIK

A Thesis
for applying for the degree of Doctor of Philosophy
in Entomology

Väitekiri
filosoofiadoktori kraadi taotlemiseks
entomoloogia erialal

Tartu 2014

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is a summary of the following papers, which are referred to by Roman numerals in the text. The papers are reproduced by kind permission of the publishers of the following journals: Biological Control (**I**), Biocontrol (**II**), Arthropod-Plant Interactions (**III**) and Crop Protection (**V**).

I **Kaasik**, **R.**, Kovacs, G., Kaart, T., Metspalu, L., Williams, I.H., Veromann, E. 2014. *Meligethes aeneus* oviposition preferences, larval parasitism rate and species composition of parasitoids on *Brassica nigra*, *Raphanus sativus* and *Eruca sativa* compared with on *Brassica napus*. Biological Control, 69, 65–71.

II **Kaasik**, **R.**, Kovacs, G., Toome, M., Metspalu, L., Veromann, E. 2014. The relative attractiveness of *Brassica napus*, *B. rapa*, *B. juncea* and *Sinapis alba* to pollen beetles. BioControl, 59(1), 19–28.

III Veromann, E., Metspalu, L., Williams, I.H., Hiiesaar, K., Mand, M., **Kaasik**, **R.**, Kovacs, G., Jogar, K., Svilponis, E., Kivimagi, I., Ploomi, A., Luik, A. 2012. Relative attractiveness of *Brassica napus*, *Brassica nigra*, *Eruca sativa* and *Raphanus sativus* for pollen beetle (*Meligethes aeneus*) and their potential for use in trap cropping. Arthropod-Plant Interactions, 6, 385–39.

IV Veromann, E., **Kaasik**, **R.**, Kovács, G., Metspalu, L., Williams, I.H., Mänd, M. 2014. Fatal attractiveness – host plant may kill its pest's larvae. Submitted.

V Veromann, E., Toome, M., Kännaste, A., **Kaasik**, **R.**, Copolovici, L., Flink, J., Kovács, G., Narits, L., Luik, A., Niinemets, Ü. 2013. Effects of nitrogen fertilization on insect pests, their parasitoids, plant diseases and volatile organic compounds in *Brassica napus*. Crop Protection, 43, 79–88.

The contribution of the author to the papers (in alphabetical order):

Paper	Idea and study design	Data collection	Data analyses	Manuscript preparation
I	GK, EV, LM, **RK**	GK, EV, LM, **RK**	GK, EV, **RK**, TK	**All**
II	GK, EV, LM, **RK**	GK, EV, LM, **RK**	EV, **RK**	**All**
III	EV, LM, **RK**	GK, EV, LM, **RK**,	EV, LM, **RK**	**All**
IV	EV, LM, **RK**	LM, **RK**	EV, LM, **RK**	**All**
V	EV, MT, LN	EV, JF, MT, **RK**	AK, EV, LC, MT	**All**

AL – Anne Luik, AK – Astrid Kännaste, AP – Angela Ploomi, ES – Eha Svilponis, EV – Eve Veromann, GK – Gabriella Kovács, IHW – Ingrid Helvi Williams, IK – Irja Kivimägi, JF – Jaak Flink, KJ – Katrin Jõgar, KH – Külli Hiiesaar, LC – Lucian Copolovici, LM – Luule Metspalu, LN – Lea Narits, MM – Marika Mänd, MT – Merje Toome, **RK** – **Riina Kaasik**, TT – Tanel Kaart, ÜN – Ülo Niinemets

INTRODUCTION

Integrated pest control was at first restricted to the combination of chemical and biological control methods (Michelbacher & Bacon, 1952) and was later widened by Stern *et al.* (1959) to "applied pest control which combines and integrates biological and chemical control". Since then the general idea has stayed the same although more than 50 definitions have been published over the years to cover all of its aspects. Today integrated pest management (IPM) is an ecological pest management approach to ensure sustainable, environmentally safe and economically viable agricultural production by utilizing diverse pest control techniques to manage the entire pest complex (Bajwa & Kogan, 2002).

The need for more sustainable pest management strategies has been underlined by environmental concerns and widespread pesticide resistance of various pests of crops worldwide. One of the tools for managing pests on agricultural land is manipulation with their preferences. The aim of this strategy, called trap cropping, is to avoid the damage in the main crop by luring pest organisms away from it during the damage susceptible time. If pest insects are concentrated on trap crop plants, the entire trap crop area can be treated with insecticides or destroyed (Hokkanen, 1991; Shelton & Nault, 2004). The development of trap cropping relies on the knowledge of pest biology together with their preferences, life cycle, natural enemies etc.

Oilseed rape (*Brassica napus* L.) is one of the most important cruciferous crops grown in Europe and other parts with a temperate climate. It is attacked by several insect pests of which the pollen beetle (*Meligethes aeneus* Fab.) is the most widespread and damaging throughout Europe. Current agricultural practices, including the management of the pollen beetle, rely heavily on chemical inputs, which have negative side effects on the environment (Alford *et al.*, 2003; Walters *et al.*, 2003). The occurrence of insecticide resistant pests has changed from "rare" to "frequent". In several European countries the pollen beetle has become resistant to a wide range of insecticides (Hansen, 2003; Heimbach *et al.*, 2006; Zaller *et al.*, 2014). To reduce dependence on chemical input and decrease the pressure on the ecosystem environmentally friendly and economically sustainable management strategies are needed.

Oilseed rape, its pests, and their natural enemies have been studied in two EU-funded Projects – BORIS and MASTER – which provided important information on several aspects of the cultivation of oilseed rape, including the potential of natural enemies to control *M. aeneus*. However, there are important knowledge gaps in tritrophic interactions between oilseed rape, their pests, and parasitoids. For instance, the feeding and oviposition preference of *M. aeneus* on crops other than oilseed rape needs to be studied so that this knowledge can be utilized in the trap crop strategy. *Brassica rapa* has been shown to be a promising trap crop for winter oilseed rape in the UK and central Europe, but there is an urgent need to find potential trap crops also for spring varieties and to test the suitability of *B. rapa* in northern countries.

1. REVIEW OF THE LITERATURE

1.1. Oilseed rape

Oilseed rape (*Brassica napus* L. Capparales: Brassicaceae) is a widely cultivated crop throughout the temperate climate (Blake *et al.*, 2010). It is mainly cultivated for its seed which is pressed for its oil used for cooking, biofuel and lubricant and also for production of soaps and synthetic rubber. The by-product of oil production is seed crust (seed-cake) which has a high protein content and is used as animal fodder for cattle and fish (Davies *et al.*, 1990; Alford, 2003). The use of oilseed rape oil to produce biodiesel has increased substantially during the last decade (Bockey, 2006).

Over recent decades, the production of oilseed rape has increased rapidly and it has become the second most important oilseed crop in Europe after sunflower (FAO, 2014) with a production of over 24 million tonnes in 2011 (Eurostat, 2014). While other oilseed crops such as sunflower and olives require higher temperatures, *B. napus* is a crop of temperate climate with growing distribution up to Sweden and Norway.

Yield formation depends on environmental factors during growth in addition to genetic characteristics of the plant variety (Sidlauskas & Bernotas, 2003). Nitrogen (N) is the most limiting nutrient for crop production (Diepenbrock, 2000) and in a temperate climate the growth and development of *B. napus* is also commonly limited by N availability (Holmes, 1980; Rathke *et al.*, 2006; Sieling & Kage, 2010). Nitrogen increases plant size, height, inflorescence branching, yield and seed protein content (Allen & Morgan, 1972; Holmes, 1980; Rathke *et al.*, 2005; Blake *et al.*, 2010; Grant *et al.*, 2010) while its deficiency decreases yield due to subsequent seed abortion (Colnenne *et al.*, 2002). The increase of the yield of *B. napus* over recent decades is the result of increased inputs of fertilizers together with plant breeding and engineering practices (Rathke *et al.*, 2006). For every ton of seed produced approximately 60–70 kg N is accumulated (Geisler & Kullman, 1991; Rathke *et al.*, 2006) while often fertilisation with mineral N exceeds the amount needed for seed development (Lickfett, 1993, 2001).

One of the main threats of excessive N fertilisation to the environment is N-leaching which often results in soil and water pollution (Di & Cameron, 2002; Sieling & Kage, 2010; Engström *et al.*, 2011). To avoid leaching from soil and its negative impact on the environment as well as to ensure economic competitiveness, only the optimum amount of N should be applied (Grant & Bailey, 1993; Aufhammer *et al.*, 1994; Shepherd & Sylvester-Bradley, 1996; Behrens *et al.*, 2001; Barlóg & Grzebisz, 2004).

The interest in N fertilisation on *B. napus* is predominantly dosage and quality related (e.g. Sidlauskas & Bernotas, 2003; Rathke *et al.*, 2005; Blake *et al.*, 2010; Grant *et al.*, 2010) although the environmental effects of N fertilisation have also been considered (e.g. Di & Cameron, 2002; Rathke *et al.*, 2006; Sieling & Kage, 2010; Engström *et al.*, 2011). However, the effects of N on pests and their natural enemies have not been studied (Rathke *et al.*, 2006).

1.2. Pollen beetle – *Meligethes aeneus* Fab.

The most abundant insect pest of cruciferous oilseed crops in Europe is the pollen beetle. Of the several species of pollen beetle found on *B. napus* (Nolte & Fritzsche, 1952; Karltorp & Nilsson, 1981), *Meligethes aeneus* Fab. (Coleoptera: Nitidulidae) is the most abundant (Alford *et al.*, 2003; Williams, 2010) in Europe. Occasionally *M. viridescens* may be abundant on spring varieties of *B. napus* (Fritzsche, 1957). However, more recent studies have shown its relative importance to be less than 2% on spring crops in northern Europe (Veromann *et al.*, 2006a, c).

Pollen beetles are univoltine. They overwinter as adults in the leaf litter, soil, herbaceous vegetation and forest borders (Büchi, 2002; Rusch *et al.*, 2011) and emerge from overwintering sites in spring when temperature reaches 10 °C (Nilsson, 1988). In spring after emerging, pollen beetles feed on the pollen of several spring-flowering plant species until the females reach sexual maturity. Beetles start to colonize cruciferous plants including oilseed rape when temperatures exceed 12 °C (Free & Williams, 1978a) to feed, mate, and oviposit (Fritzsche, 1957; Williams & Free, 1978a; Ekbom & Borg, 1996). Females oviposit in 2–3 mm long flower buds (Nilsson, 1988; Borg, 1996). Beetles feed on buds and cause bud abscission (Free & Williams, 1978a) during the early bud stage,

when plants are at their most susceptible (Williams & Free, 1979; Nilsson, 1987, 1988). Feeding by adults during the bud stage of plants can cause yield reduction up to 70% in untreated spring oilseed rape (Nilsson, 1987). They occasionally can cause damage also on winter varieties of *B. napus* but their life cycle is better synchronised with spring varieties (Williams, 2004; Veromann *et al.*, 2006c; Williams, 2010). From the start of flowering, both larvae and adults have easy access to pollen from open flowers and hence only little further damage is caused (Williams & Free, 1978a). New generation beetles emerge in late summer and feed polyphagously before migrating to hibernation sites (Williams & Free, 1978a).

The most common method to manage *M. aeneus* is based on synthetic insecticides (Walters *et al.*, 2003; Williams, 2004; Thieme *et al.*, 2010). As these are easily available and affordable they are often used routinely and prophylactically, regardless of pest incidence (Alford *et al.*, 2003; Williams, 2004; Thieme *et al.*, 2010). This has led to widespread pyrethroid resistance of *M. aeneus* (Hansen, 2003; Heimbach *et al.*, 2006; Cook & Denholm, 2008; Hansen, 2008; Tiilikainen & Hokkanen, 2008; Thieme *et al.*, 2010; Zaller *et al.*, 2014). In Europe, 80% of oilseed rape crops are always treated with insecticides (Menzler-Hokkanen *et al.*, 2006). In addition, Hokkanen (2000) has shown that insecticide treatment at the threshold level does not reduce population size of the new generation of pollen beetles and Veromann *et al.* (2008) found that it even increased it. Such routine use of pesticides increases the selection of insecticide resistance of the pest in addition to having negative effects on the environment, biodiversity and on non-target or beneficial organisms.

1.3. Parasitoids of *Meligethes aeneus*

Meligethes aeneus is parasitized by at least nine species of parasitoids which all attack during the larval stage. All known parasitoids belong to the order Hymenoptera: *Aneuclis incidens* Thomson, *Phradis interstitialis* Thomson, *P. morionellus* Holmgren and *Tersilochus heterocerus* Thomson to the family Ichneumonidae; *Blacus nigricornis* Haeselbarth, *Diospilus capito* Nees and *Eubazus sigalphoides* Marshall to the family Braconidae; *Cerchysiella planiscutellum* Mercet to the family Encyrtidae and *Brachyserphus parvulus* Nees to the family Proctotrupidae (Nilsson, 2003).

The parasitism rate of *M. aeneus* can reach up to 90% (Ulber *et al.*, 2006) although usually it varies from 25% to 50% (Ulber *et al.*, 2010). However, in conventional cropping systems it can be even below 4% (Veromann *et al.*, 2009). A parasitism rate of 30–40% can effectively manage *M. aeneus* abundance (Hokkanen, 2008).

The most important species in conservation biocontrol are *P. interstitialis*, *P. morionellus*, *T. heterocerus* and *D. capito*, these species are distributed throughout Europe (Nilsson, 2003; Williams *et al.*, 2005; Ulber *et al.*, 2006; Ferguson *et al.*, 2010; Ulber *et al.*, 2010). In winter varieties of *B. napus*, *T. heterocerus* and *P. morionellus* are dominant (Ulber & Nitzsche, 2006; Williams, 2006; Ulber *et al.*, 2010). Species of the genus *Phradis* and *Tersilochus* are univoltine whereas *D. capito* is multivoltine. Most larval endoparasitoids develop in the host's pupal chamber, they spin a cocoon in the cavity prepared by *M. aeneus* larvae (Osborne, 1960). Multivoltine *D. capito* can emerge from the soil already after 10 days (Osborne, 1960). The pupal stage of univoltine species lasts about a month but the adult parasitoid stays in diapause in the cocoon until next spring or summer. The emergence of univoltine species is synchronised with the flowering time of the variety of *B. napus* on which its host was feeding. If the parasitoid eggs were laid in a pollen beetle that was feeding on winter oilseed rape flowers then parasitoid adults will emerge when winter oilseed rape is flowering and similarly, if the parasitoid developed in *M. aeneus* larvae feeding on spring oilseed rape, it will emerge when spring oilseed rape is flowering (Nilsson, 2003). Females are able to oviposit immediately after emerging from their overwintering places as they arrive at the crop seeking suitable host larvae. Females of *T. heterocerus* usually lay eggs in second-instar larvae (Nilsson & Andreasson, 1987) as probably does *P. morionellus*. They can be found at the beginning of the flowering stage of oilseed rape plants where they seek hosts in open flowers. However, parasitoid appearance and crop development are not synchronized precisely (Nilsson, 1985). Osborne (1960), Winfield (1963) have shown that adult *P. interstitialis* appear in the field earlier to seek hosts also from buds as they can bore their ovipositors through the bud wall and lay eggs into the first-instar larvae or eggs of pollen beetle. Nitzsche and Ulber (1998) have reported that in case of competition between *P. interstitialis* and *T. heterocerus* in a host larva, the winner is *P. interstitialis*, probably because its eggs hatch before its competitor's eggs. Neither *P. interstitialis* nor *T. heterocerus* discriminate between parasitised and non-parasitised hosts, they lay their eggs randomly. Females of *D. capito* do not have

preferences for the size of hosts and they lay their eggs into all instar stages of hosts including eggs (Osborne, 1960). The larva has strong mandibles and can kill other parasitoid larvae in the same host body; therefore they are superior in interspecific competition (Jourdheuil, 1960). *Diospilus capito* gains more importance on spring varieties of oilseed rape because the next generation is more numerous than the overwintered generation. It is a very important parasitoid species in northern Europe (Miczulski, 1967; Nilsson, 2003; Veromann *et al*., 2006b, c; Hokkanen, 2008).

1.4. Host location by *Meligethes aeneus* and its parasitoids

Insects use a variety of cues for host plant location, of which chemical and visual stimuli are the most studied. The majority of herbivorous insects probably use both olfactory and visual cues although which are more important is still debated (Finch & Collier, 2000). Chemical stimuli are probably more informative from a distance although they can also be used at close range (Finch & Collier, 2003; Couty *et al*., 2006). Visual cues are mainly studied in flying insects and are more related to close-range host plant selection (Finch & Collier, 2000; Kühnle & Müller, 2011).

For host plant location, *M. aeneus* uses a combination of olfactory and visual cues. *Meligethes aeneus* has colour specific preferences and is attracted to the colour which is recognized as 'yellow' by humans over colours such as 'blue', 'red', 'black', 'green' or 'white' (Giamoustaris & Mithen, 1996; Blight & Smart, 1999; Cook *et al*., 2006b; Döring *et al*., 2012; Cook *et al*., 2013). The vision of insects is fundamentally different from that of humans (Chittka & Döring, 2007) who have blue, green and red (which also responds to yellow) receptors for photopic vision (Wyszecki & Stiles, 1982; Dartnall *et al*. 1983) while the majority of insects have green, blue and ultraviolet receptors (Briscoe & Chittka, 2001). *Meligethes aeneus* prefers 'canary yellow' over 'yellow-green' and 'fluorescent yellow' over 'yellow' in field conditions (Blight & Smart, 1999; Döring *et al*., 2012) although the colour beetles see is caused by a positive input from their green receptors and a negative one from the blue receptors (Döring *et al*., 2012).

The use of olfactory cues by *M. aeneus* has also been studied. They reach the host plants flying upwind to the source of attractive odour (Evans & Allen-Williams, 1994). A wide range of volatile organic compounds

(VOCs) are attractive to *M. aeneus*, emitted by both cruciferous and non-cruciferous plants (Free & Williams, 1979; Charpentier, 1985; Ruther & Thiemann, 1997; Blight & Smart, 1999; Cook, 2000). Their allurement of diverse volatiles relies on beetles' oligophagous feeding. A variety of VOCs attractive to *M. aeneus* have been identified, such as several amino and fatty acid derivates (Smart, 1995; Smart & Blight, 2000; Cook *et al.*, 2007b). They are attracted to the odour of *B. napus* and *B. rapa* in bud as well as in the flowering stage (Evans & Allen-Williams, 1994; Ruther & Thiemann, 1997; Cook *et al.*, 2006a) and both floral volatiles and isiothiocyanates have an influence on the abundance of *M. aeneus* (Smart & Blight, 2000; Cook *et al.*, 2002; Evans & Allen-Williams, 1994).

Herbivorous insects are affected directly and indirectly by plant nutritional value which depends on fertilisation (reviewed in Chen *et al.*, 2010). Nitrogen deficiency is one of the most limiting factors for *B. napus* growth and development in temperate climates (Holmes, 1980; Rathke *et al.*, 2006; Sieling & Kage, 2010). Insects are directly affected by plant size and architecture (Walters *et al.*, 2003; Ulber & Fisher, 2006). Nitrogen fertilisation also affects the glucosinolate content of plants (Josefsson, 1970). Nitrogen availability may also have a significant effect on the composition and levels of VOCs (Chen *et al.*, 2010) and therefore may influence pest and parasitoid host location as they both use volatile cues to locate host plants (Bernays & Chapman, 1994; Hilker & McNeil, 2008).

1.5. Sustainable plant protection strategies

Modern intensive agriculture has been developed at the field scale to produce high quantity and quality yields; the input to this system relies heavily on non-reproducible sources to produce energy, fertilizers and pesticides to control pests and diseases. The over-simplification of the modern agro-ecosystems has increased the damage by pests and diseases (Tilman *et al.*, 2002), which are often handled with increased inputs of synthetic pesticides. From the 1950s to 1980s the use of conventional insecticides was the main pest control method. The severe side-effects to human health and environment, increased problems with insecticide resistance, secondary pest outbreaks have raised the need for alternative ecologically more sustainable pest control methods. Integrated pest management (IPM) started as a suggestion to combine chemical and biologi-

cal control methods (Michelbacher & Bacon, 1952) and was developed further with suggestion to monitor pest abundance prior to insecticide treatment (Stern *et al*., 1959). Later the term 'IPM' was widened to include coordinated use of all biological, cultural, and artificial practices (van den Bosch and Stern, 1962). Today IPM combines different management strategies and practices to manage the entire pest complex for minimizing pesticide use in crop production to ensure sustainable, environmentally safe and economically viable agricultural production (Bajwa & Kogan, 2002).

One of the sustainable pest management strategies is trap cropping where plants are grown in proximity to the main crop to attract pest organisms, to prevent their colonisation of the crop and/or retain targeted pests to prevent or reduce potential damage (Hokkanen, 1991; Shelton & Badenes-Perez, 2006). Trap crops which involve manipulation with insect preferences can be classified according to their utilisation: i) to avoid the damage by redirecting them away from the crop of interest – conventional trap cropping; ii) luring pests to plants which do not support their development – dead-end trap cropping and iii) attracting natural enemies to the field – biological control-assisted trap cropping (Landis *et al*., 2000; Shelton & Nault, 2004; Cook *et al*., 2006a; Shelton & Badenes-Perez, 2006; Cook *et al*., 2007a).

Conventional trap cropping

Conventional trap cropping is based on insect preferences and their host location abilities as insects use both visual and olfactory cues to locate suitable host plants for feeding and oviposition. Knowledge of their preferences can be used in plant protection practices (Hokkanen, 1991; Shelton & Badenes-Perez, 2006) in several cropping systems (De Sahagun, 1969; Talekar & Shelton, 1993; Godfrey & Leigh, 1994). To manage *M. aeneus* on winter variety of *B. napus, B. rapa* has shown promising results (Cook *et al*., 2007b). Trap crops are only effective if they are highly attractive to the pest either due to plant species, cultivar or more suitable growth stage. A more advanced and complex way of trap cropping is the push-pull strategy, which uses repellent sources to deter pests from the main crop additionally to an attractive trap crop element to attract pests (Cook *et al*., 2007a).

Dead-end trap cropping

The pest management strategy using plant species that are highly attractive to pests for oviposition but which do not support the development of the larvae is called dead-end trap cropping (Shelton & Nault, 2004; Shelton & Badenes-Perez, 2006). The suitability of the plant for this strategy can be achieved via the intrinsic characteristics of a plant species, such as nutritional ineligibility, chemical defence or even introduced insecticidal genes such as *Bacillus thuringiensis* Berliner (Lu *et al.*, 2004; Cao *et al.*, 2005; Badenes-Perez *et al.*, 2005).

Dead-end trap cropping has mainly been studied for lepidopteran pests (Van der Berg *et al.* 2003; Lu *et al.*, 2004; Shelton & Nault, 2004; Cao *et al.*, 2005; Shelton & Badenes-Perez, 2006) but also for Colorado beetle (Hoy, 1999) and nematodes (Melakeberhan *et al.*, 2006).

Biological control-assisted trap cropping

Parasitoids and predators gain from plant-provided resources as they obtain a significant amount of their nutrition from plants. Pollen, nectar and plant sap are important energy sources and are utilized by both parasitoids and predators (Landis *et al.*, 2000, 2005; Wäckers, 2005). While searching the host, parasitoids are also highly plant dependent as they rely more on cues from plants than from their hosts themselves as the former are more informative due to greater biomass (Godfray, 1994). In the case of biological control-assisted trap cropping natural enemies gain from the plant species used as a trap crop. Therefore, by selecting plant species based on their attractiveness, resource provision and quality, biological control by parasitoids can be enhanced (Billqvist & Ekbom, 2001, Khan & Pickett, 2004; Virk *et al.*, 2004).

2. HYPOTHESIS AND AIMS OF THE STUDY

Herbivorous insects are classified as specialist, oligophagous or generalist feeders through their host plant specialisation level, which varies from one plant species to a wide range of plants (Bernays & Chapman, 1994). Phytophagous insects use visual and olfactory cues to locate suitable host plants. *Meligethes aeneus* is an oligophagous species; it feeds on pollen from different plant families in spring and autumn but oviposits only in the buds of cruciferous plants (Fritzsche, 1957; Free & Williams, 1978; Alford *et al.*, 2003; Williams, 2010). They are particularly attracted to yellow colour and host plant odour (Evans & Allen-Williams, 1994; Giamoustaris & Mithen, 1996, Cook *et al.*, 2002). Crucifer specialist insects and their parasitoids are especially attracted to isiothiocyanates (Blight & Smart, 1999; Smart & Blight, 2000; Alford *et al.*, 2003). Nitrogen fertilisation affects glucosinolate content in plants (Josefsson, 1970) and has a great impact on the composition and levels of VOCs (Chen *et al.*, 2010) therefore influencing pest and parasitoid host location.

Hypothesis:

1. The attractiveness of cruciferous plants to oligophagous *M. aeneus* adults for feeding differs with plant species.

2. Cruciferous plants differ in their attractiveness for oviposition to *M. aeneus* and its parasitoids.

3. The developmental success of *M. aeneus* larvae depends on plant species; not all plant species chosen by *M. aeneus* for oviposition support their development during the larval stage.

4. Nitrogen fertilisation affects the abundance of *M. aeneus* and its parasitoids. These modifications are associated with the emission of VOC.

The aims of this study were:

1. To determine the potential plant species suitable for trap cropping strategy by comparing the relative attractiveness of different cruciferous plant species for feeding and oviposition to manage *M. aeneus* on spring oilseed rape (**I**, **II**, **III**, **IV**).

2. To determine whether host plant species influence the host finding success of *M. aeneus* parasitoids and/or their species composition (**I**, **II**).

3. To determine the oviposition preferences and larval survival rate of *M. aeneus* on different cruciferous plant species to develop dead-end trap cropping (**IV**).

4. To determine if and how fertilisation with mineral nitrogen influences *M. aeneus* and its hymenopteran parasitoids and whether these are associated with the emission of volatile organic compounds (**V**).

3. MATERIAL AND METHODS

3.1. Study area and experimental design

Studies were carried out in an experimental field of the Estonian University of Life Sciences during summers 2006 to 2012 (**I**, **II**, **III**, **IV**). Over the years, seven cruciferous plant species were compared: *B. napus*, *Sinapis alba*, *Eruca sativa*, *Raphanus sativus*, *B. nigra*, *B. rapa* and *B. juncea* (Capparales: Brassicaceae). The experiments were laid out in randomised complete block design with three replicates of each plant species. All plots were 1 × 5 m with a 1 m wide buffer zone around each plot to minimize inter-plot interactions; the same width of buffer zone surrounded the whole experiment, which, in turn was surrounded by non-cruciferous vegetable experiments (potato, beetroot, carrot etc.) in 2006, 2007 and 2008; spring barley in 2009 and 2010 and red clover in 2011 and 2012.

Seeds for experiments were obtained either from the seed collection of the Estonian University of Life Sciences or purchased from Hansaplant LLC seed company (Tartu, Estonia). Over the years, the same cultivars of all plant species were used: *B. napus* cv 'Mascot', *E. sativa* cv 'Poker', *R. sativus* 'Bille', *S. alba* 'Braco'. Neither pesticides nor fertilizers were applied during the study.

The effects of nitrogen fertilisation on *M. aeneus* and its parasitoids at seven different nitrogen (N) levels (0, 60, 80, 100, 120, 160 kg of N ha^{-1} (applied as NH_4NO_3)) were studied in 2008 and 2009 in an experimental field of Jõgeva Plant Breeding Institute, Estonia using winter oilseed rape cv 'Silva' (**V**). The experiment was laid out in three randomized replicate plots of 10 m^2. Fertiliser at different N levels was applied on 23 April each year. Neither insecticides nor fungicides were applied.

Plant growth stages (BBCH) were determined using the decimal code system of Lancashire *et al.* (1991): 0–9 germination, 10–19 leaf development, 20–29 formation of side shoots, 30–39 stem elongation, 50–59 inflorescence emergence, 60–69 flowering, 70–79 development of fruit, 80–89 ripening, 90–99 senescence.

3.2. Insect sampling

The abundance and feeding preferences of adult beetles were assessed using an aspirator during BBCH 12–39 (**III**) and by using the beating method (Williams *et al*., 2003) during BBCH 23–67 of *B. napus* (**II**) and during BBCH 50–69 of all plant species (**III**).

Oviposition preferences and larval abundance were determined at the full flowering of plants (BBCH 65–68) using the flower and bud dissection method (**I**, **II**, **III**, **IV**, **V**) and from the inflorescence emergence until the end of flowering (BBCH 50–69) using the beating method (**IV**).

The abundance of *M. aeneus* eggs was determined by dissecting green (BBCH 51–53) (**III**, **IV**) and yellow (BBCH 58–59) (**IV**) buds.

Oviposition preferences and survival rate of *M. aeneus* larvae on buds of different plant species (**IV**) were assessed by dissecting green and yellow buds (BBCH 51–59) and counting the eggs and dead or live *M. aeneus* larvae found.

To estimate parasitization level (**I**, **II**, **V**), second instar larvae of *M. aeneus* collected from the flowers during full flowering (BBCH 65–68) were dissected under the microscope (Olympus SZ-CTV) and parasitoid eggs and larvae were identified to species level using the key of Osborne (1960).

3.3. Statistical analysis

The abundance of adult *M. aeneus* per sampling time and plant species were analysed using GLM and GENMOD procedure with Wald statistic Type III empirical standard error analysis with Poisson distribution and *log* link function (**II**) and analysis of variance (ANOVA) and LSD post hoc test (**III**).

The significance of plant species and year on the abundance of *M. aeneus* larvae (**I**, **II**, **III**, **V**) and eggs and larvae (**IV**) on different plant species, were studied using GLIMMIX Procedure with year and plant species as fixed (**I**), GLM and GENMOD Procedure with Wald statistic Type III empirical standard error analysis with Poisson distribution and *log* link

function (**II**, **V**) and SAS 9.1 (**IV**), ANOVA with LSD post hoc test in (**III**).

The comparisons of buds infected by *M. aeneus* were made with GENMOD Procedure with Wald statistic Type III empirical standard error analysis with binomial distribution and *logit* link function (**IV**).

The significance of plant species on the parasitism rate of *M. aeneus* was analysed with GLIMMIX Procedure considering plant species as fixed effect (**I**) and with GENMOD Procedure with Wald statistic Type III empirical standard error analysis with binomial distribution and *logit* link function (**II**).

The flower supply was analysed using GLIMMIX Procedure considering plant species as fixed effect (**I**). Correlations between flower supply, larval abundance and parasitism rate and emission rates of volatiles from buds were analysed using Spearman (**I**, **V**) and Pearson correlation (**II**). The relationships between nitrogen treatment and abundance of *M. aeneus* larvae and its parasitoids were analysed using Spearman correlation (**V**).

Parasitoid species composition on different plant species was compared using the Fisher's exact test (**I**).

The dependence of VOC emissions on nitrogen fertilisation was analysed using non-linear regression model in the exponential form. The bouquets of volatiles emitted at bud and flowering stage were explored using principal component analysis and the differences were tested using Monte-Carlo permutation test using redundancy data analysis (**V**).

Statistical analyses were carried out using statistical software programs SAS (SAS Institute, INC., Cary, NC, USA) versions 8.02 (**II**, **IV**) and 9.1 (**I**, **IV**), Statistica (StatSoft, Inc) version 9.1 (**III**), OriginPro 8 (OriginLab Corporation, Northhampton, MA, USA) (**V**) and Canoco 4.5 (ter Braak and Smilauer, Biometrics Plant Research International, The Netherlands) (**V**). All statistical tests were considered statistically significant at $P < 0.05$.

4. RESULTS

4.1. The impact of host plants on the abundance of *Meligethes aeneus* adults

Over the study, *M. aeneus* adults were found from all studied plant species (Fig. 1) but plant species had significant influence on their abundance (II: χ^2 = 62.57, df = 3, P < 0.0001; III: Kruskal-Wallis ANOVA KW-H(3, 234) = 13.40, P < 0.0038). More beetles were found on *B. nigra* and *S. alba* (**II**, **III**) than on *B. napus*. The abundance of *M. aeneus* on *B. napus* was similar to that on *B. rapa*, *B. juncea, E. sativa* and *R. sativus* (**II**, **III**).

Sinapis alba and *B. nigra* were more attractive plant species for *M. aeneus* during the damage susceptible growth stage (green and yellow bud growth stage (BBCH 51–59)) of *B. napus* (Fig. 2) (**II**, **III**). Beetles were more attracted to *B. napus* from the start of flowering (BBCH 60) (**II**, **III**). Plant species had a significant impact on the colonization rate of pollen beetles at the green and yellow bud growth stage of plants (BBCH 51–53 and 55–59, respectively). From full flowering (BBCH 65) until the end of flowering (BBCH 69) plant species did not influence the abundance of beetles (**III**).

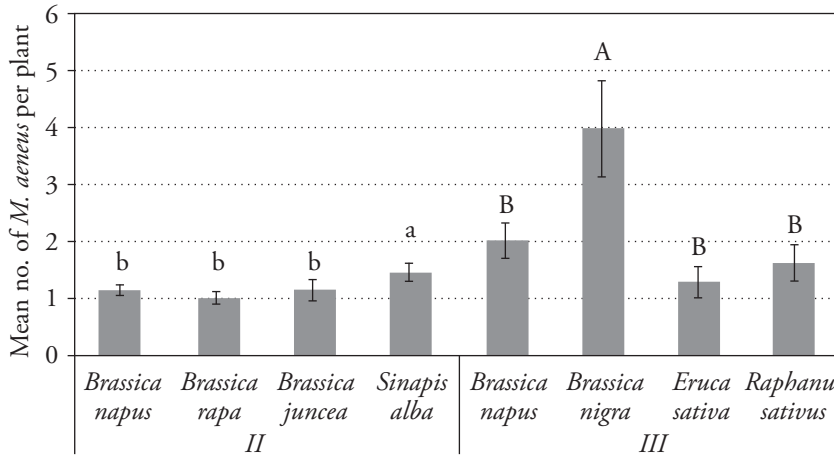

Figure 1. The mean (±SE) number of *Meligethes aeneus* adults per main raceme 2006–2008 (II) and 2009–2010 (III). Different letters indicate significant differences between plant species, lowercase letters – II, capital letters – III (P < 0.05).

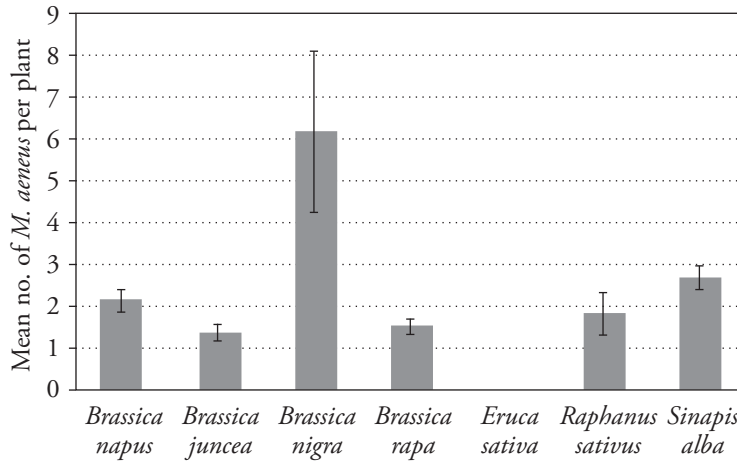

Figure 2. The mean (±SE) number of adult *Meligethes aeneus* per plant during the damage susceptible stage (BBCH 50–59) of *Brassica napus* 2006–2010.

4.2. The influence of host plants on the oviposition preferences and larval survival rate of *Meligethes aeneus*

All studied plant species were used for oviposition by *M. aeneus*, but the host plant species had significant influence on preferences (Fig. 3) (**I**, **II**, **III**, **IV**). During the bud stage (BBCH 51–59), *M. aeneus* preferred to oviposit on Brassica species over *E. sativa* and *S. alba* (**III**, **IV**) but not over *R. sativus* (**IV**). Oviposition preferences were similar in green and yellow bud stage (**IV**). *Brassica nigra* was equally or more preferred to other plant species for oviposition (**III**, **IV**).

During full flowering (BBCH 67–69), larval abundance on Brassica species was higher than on *E. sativa* and *S. alba* (**I**, **II**, **III**). The differences were detected among the genus Brassica as well. In general, *B. napus* was more attractive than *B. rapa* and *B. juncea* (**II**).

Over the years, *B. nigra* varied in attractiveness to pollen beetles: in 2009 and 2010 it was favoured over all studied plant species (**III**) while data

pooled over the years also showed similar oviposition preferences of *M. aeneus* on *B. nigra* and *B. napus* (**I**). The attractiveness of *R. sativus* also varied between years (**I**, **III**), the number of larvae in dissected flowers was also greater on *B. nigra* than on all other studied plant species in 2009 and 2010 (**III**). However, when the data was pooled (2009–2011) there was no difference between *B. nigra* and *R. sativus* (**I**). Over the studies higher or similar abundance was found on *B. nigra* (**I**, **III**) and lower or similar on *R. sativus* compared to *B. napus* (**I**, **III**).

The size of *M. aeneus*' egg clutch also depended on plant species at both green and yellow bud stages (**IV**). Clutch sizes were bigger on genus Brassica than on other plant species. More eggs and larvae per bud were found on *R. sativus* than on *E. sativa* and *S. alba*. The biggest clutch size was on *B. nigra* (2.3±0.04 specimens per bud) which was significantly larger than on *B. napus*. Compared to *B. napus*, fewer eggs were laid in the buds of *E. sativa, R. sativus* and *S. alba*.

The survival rate of *M. aeneus* larvae depended on plant species as well; dead larvae were found only on *R. sativus* and *E. sativa,* but the mortality rate was significantly higher on *R. sativus* (**III**). Year as a factor had no

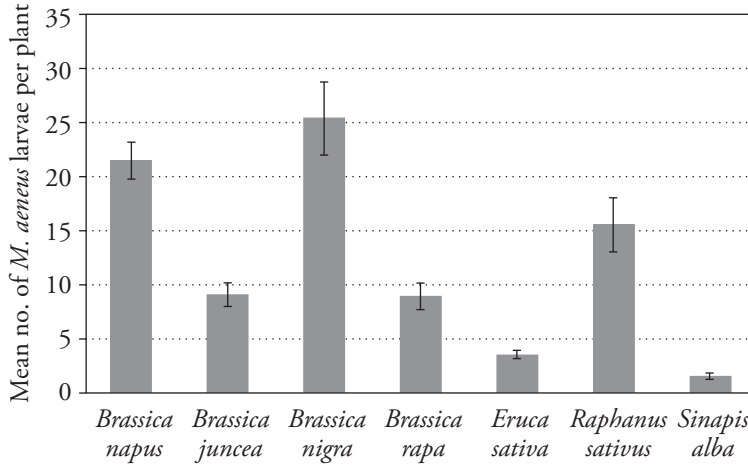

Figure 3. The mean (±SE) number of *Meligethes aeneus* larvae on different host plant species 2006–2011.

influence on the abundance of dead larvae. Over the years, on *R. sativus* 35% and on *E. sativa* 19% of the larvae were dead. On *E. sativa* larvae died shortly after hatching in green buds while on *R. sativus* larvae failed to complete the moulting process.

4.3. Parasitism rate of *Meligethes aeneus* on different plant species and its dependence on host availability

In total, 4666 larvae were dissected during the study years (**I**, **II**). The mean parasitism rate of *M. aeneus* over the years was relatively high (36.5%), varying from 20% up to 58% between the years.

The parasitism rate of *M. aeneus* depended on plant species and varied from 5% on *R. sativus* up to 65% on *B. juncea* over the whole study period (Fig. 4) (**I**, **II**). Compared to *B. napus* the parasitism rate was higher on *B. juncea* and *B. nigra* (**I**, **II**), lower on *E. sativa* and *R. sativus* (**I**) and similar on *B. rapa* and *S. alba* (**II**). On most plant species the parasitism rate was density independent (**I**, **II**) however, on *R. sativus* moderate correlation between host abundance and parasitism rate was found (**I**).

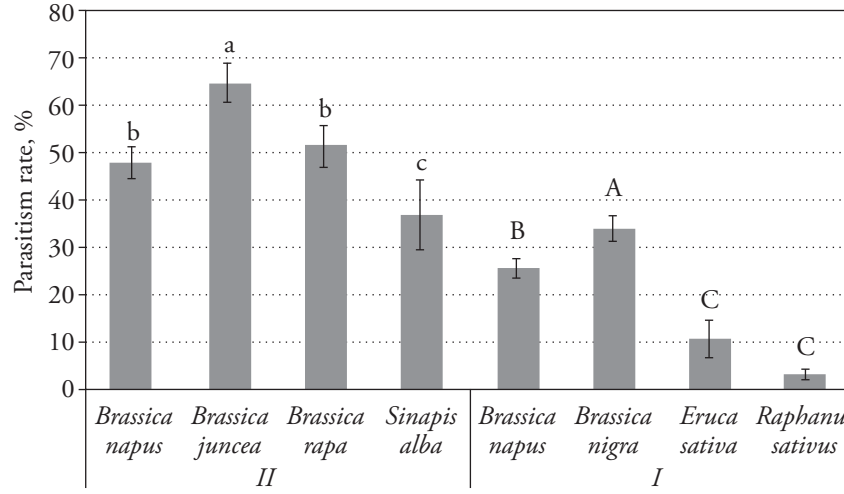

Figure 4. The mean (±SE) parasitism rate of *Meligethes aeneus* larvae on different host plant species 2006–2008 (II) and 2009–2011 (I). Different letters indicate significant differences between plant species, lowercase letters – II, capital letters – I ($P < 0.05$).

4.4. Species composition of parasitoids of *Meligethes aeneus* on different host plants

In total, during the study 1430 specimens of hymenopteran parasitoids were found from *M. aeneus* larvae collected from flowers of annual cruciferous oilseed crops (**I**, **II**). The greatest number of parasitoids was found from larvae collected from *B. napus* (46%) and *B. nigra* (34%); fewer than 10% of parasitoids were found from the other plant species (**I**, **II**).

Parasitoids of four species: *T. heterocerus*, *P. morionellus*, *D. capito* and one unidentified parasitoid, named 'healthy-fat-one' were found (**I**, **II**). All species but the unidentified one were present each year of the study. The most abundant parasitoid species was *T. heterocerus* (608 specimens), followed by *P. morionellus* (452 specimens) and *D. capito* (364 specimens) (Fig. 5) (**I**, **II**). The unidentified species was very rare as over the 6 years only 6 specimens were found all from *B. napus* or *R. sativus* (**I**).

Parasitoid species composition varied between plant species; *T. heterocerus* as the most abundant parasitoid species during the study, mainly achieved its status due to its high occurrence on *B. nigra*, where it constituted over 75% of all parasitoids. Contrary to *B. nigra* where one species significantly prevailed over others, the species ratio on *B. napus* was relatively

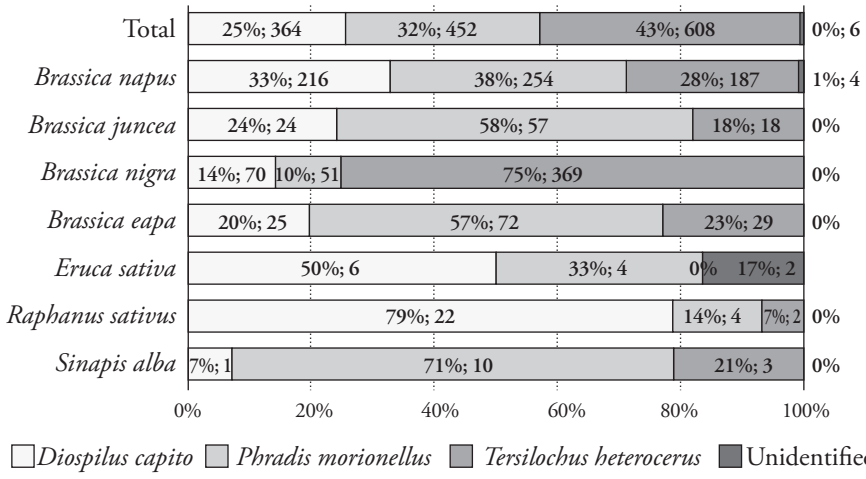

Figure 5. Species composition, percentage and total number of *Meligethes aeneus* larval endoparasitoids on different plant species in 2006–2011.

similar, varying from 28% to 38%. Also, species ratio was rather similar on *B. juncea* and *B. rapa*, where the dominating species was *P. morionellus*. Parasitoid species composition on host plants not belonging to the Brassica family differed from each other. In total, *D. capito* was the most abundant species, followed by *P. morionellus*. *Tersilochus heterocerus* was not present on *E. sativa*.

4.5. Impact of nitrogen fertilisation and emitted volatiles of *Brassica napus* on the abundance of *Meligethes aeneus* larvae and its parasitism rate

Oviposition preferences of *M. aeneus* and its parasitoids were also affected by the fertilisation level. Fertilisation with nitrogen at 60, 80 and 160 kg N per hectare were more preferred by *M. aeneus* (Fig. 1a in **V**, pg. 83). The parasitoid complex on the other hand preferred *M. aeneus* larvae on patches where no additional nitrogen was added; there the parasitism rate was 26%. The number of *M. aeneus* larvae correlated with the abundance of parasitized larvae.

From 19 different VOCs detected, acetic acid, 3-carene, α-pinene and benzaldehyde had the highest emissions. Volatile bouquets from bud and flower stages were different, several compounds were emitted at lower levels during the flowering stage, especially lipoxygenate pathway volatiles (LOX) (Table 2 in **V**, pg. 84). During flowering, plants fertilized with higher amounts of nitrogen clustered separately from the control (Table 2 in **V**, pg. 84). Increased nitrogen availability increased the emission of LOX and acetic acid at both bud and flowering stages, while the emission of α-thujene was increased only during flowering stage. There were only two compounds, methyl salicylate and acetic acid, that were produced at increased levels at greater N levels both at bud and flowering stages.

The emissions of several volatiles during the bud stage were correlated with the abundance of *M. aeneus* larvae (Table 3 in **V**, pg. 86). Significant positive correlations were found between *M. aeneus* abundance and emission rates of (3Z)-hexenyl acetate, camphene, 3-carene, limonene, indole and acetic acid. The correlations were negative for β-pinene, linalool, (E,E)-α-farnesene, benzaldehyde and methylbenzoate emission rates.

5. DISCUSSION

5.1. Manipulation with beetles' preferences to find potential trap and dead-end trap crops for oilseed rape

The potential of i) *B. nigra* and *S. alba* for conventional trap cropping to avoid the damage caused by *M. aeneus* on spring *B. napus* (**II**, **III**); ii) *R. sativus* as a dead end trap crop to reduce the population of *M. aeneus* by killing the larvae (**IV**) and iii) *B. juncea* and *B. nigra* as biological control-assisted trap crop for *M. aeneus* by supporting its natural enemies (**I**, **II**) are shown for the first time.

To date research on trap cropping strategy for control of *M. aeneus* has concentrated on the development of conventional trap cropping and a push-pull strategy using *B. rapa* and a low isothiocyanate variety of *B. napus* (Hokkanen *et al.*, 1986; Buechi, 1990, 1995; Cook *et al.*, 2002, 2006a, Valantin-Morison & Quere, 2006; Cook *et al.*, 2007b, 2008; Hokkanen, 2008; Valantin-Morison & Meynard, 2012). Our study has investigated the relative attractiveness to *M. aeneus* of different plant species compared to that of a spring variety of *B. napus*.

5.2. Feeding preferences of *M. aeneus*

All studied plant species were suitable for overwintered *M. aeneus* adults for feeding. However, *B. nigra* and *S. alba* were preferred over *B. napus* and *E. sativa* was unattractive for them over the whole study period. Plant species suitable for trap cropping have to be attractive to the pest, but the basis of greater attractiveness can differ. Higher attractiveness can be caused by plant species, variety or the growth stage (Hokkanen, 1991).

Sinapis alba was more attractive than *B. napus*, probably due to its plant characteristics. The flowers of *S. alba* are more abundant, larger and closer together than those of *B. napus* (Ekbom & Borg, 1996). The attractiveness of the colour yellow to *M. aeneus* has been shown in several studies (Giamoustaris & Mithen, 1996; Blight & Smart, 1999; Cook *et al.*, 2006a, Döring *et al.*, 2012).

The attractiveness of plant species changed over the vegetation period. The development of most plant species was faster than that of *B. napus.* Over the study the growth stages of *B. rapa, B. juncea* and *S. alba* were similar but they were not equally attractive to *M. aeneus*: coincidence of growth stages even in the case of *B. rapa* and *B. juncea* did not lead to similar attractiveness. Previous studies have shown the importance of the growth stage of the host plant to *M. aeneus*, for example, more advanced growth stages are preferred in the case of *B. napus* and *B. rapa* (Free & Williams 1978b; Ferguson *et al.* 2003; Frearson *et al.* 2005; Cook *et al.*, 2007b). In the current studies more advanced growth stages of *B. rapa*, *B. juncea, R. sativus and E. sativa* compared to that of *B. napus* did not lead to higher attractiveness (**II**, **III**). Only *B. nigra*'s slightly more advanced growth stage led to a significantly greater attractiveness. The preference of *B. nigra* over *B. napus* also remained during the same growth stage (**III**). Beetles probably choose more advanced growth stages during bud and flowering searching for potential feeding and oviposition sites.

The most yield limiting damage caused by *M. aeneus* on *B. napus* takes place during the bud stage (Williams & Free, 1979; Nilsson, 1994) whereas feeding from open flowers causes little damage (Williams & Free, 1978). Therefore, a conventional trap crop should be highly attractive to *M. aeneus* primarily during the bud stage and not necessary over the whole vegetation period. In the current study *B. nigra* and *S. alba* were clearly more attractive throughout the bud stage as nearly twice as many beetles were found on them as on *B. napus*. These results suggest that plants would be suitable for conventional trap cropping to avoid damage caused by *M. aeneus* feeding on *B. napus* (**II**, **III**).

Although previous studies have shown a greater attractiveness of *B. rapa* over *B. napus* for *M. aeneus* in case of winter varieties (Buechi, 1990; Valanin-Morison & Quere, 2006; Rusch *et al.*, 2010; Valantin-Morison & Meynard, 2012) and on one year on a spring variety as well (Cook *et al.*, 2006a) in the current study the attractiveness of spring sown *B. rapa* did not exceed that of *B. napus*. The number of beetles per plant was similar during the damage susceptible stage of *B. napus* and was even lower during the beginning of its flowering stage, when *B. rapa* was in full flower (**II**). These results concur with Cook *et al.* (2007b) who hypothesised that early flowering odour cues may provide reliable cues for the availability of the buds and therefore attract female beetles.

Despite the characteristic differences between *B. nigra* and *S. alba* they both were preferred over *B. napus* by *M. aeneus* during the damage-susceptible phase of *B. napus* which proves their attractiveness for overwintered *M. aeneus* adults probably for feeding. These results indicate that *B. nigra* and *S. alba* can serve as earlier food sources to *M. aeneus* and hence prevent the damage caused by their feeding on the buds of *B. napus*.

5.3. Oviposition preferences of *M. aeneus* and its parasitoids

Brassica napus was clearly more attractive for oviposition by *M. aeneus* than *B. juncea*, *B. rapa*, *E. sativa*, *R. sativus* and *S. alba* (**I**, **II**, **III**); this partially confirms previous studies by Ekbom and Borg (1996) and Ekbom (1998) who also found more *M. aeneus* larvae from *B. napus* compared to *S. alba* and *E. sativa*. The attractiveness of *R. sativus* was equal to several Brassica species in contrast to previous studies in which plant species from genus Brassica were preferred over *R. sativus* (Ekbom, 1998).

Larval abundance in the flowers of *B. nigra* and *B. napus* differed between studies (**I**, **III**). In some years, *B. nigra* was highly preferred over *B. napus* for oviposition (**III**) while their attractiveness can also be at the same level (**I**). Thus current results contrast with previous studies by Ekbom and Borg (1996), Ekbom (1998) and Ulber and Thieme (2007) who found *B. napus* was preferred over *B. nigra* by *M. aeneus* for oviposition. We conclude, that *B. nigra* has potential as a trap crop for *M. aeneus* as it might diminish the oviposition pressure on *B. napus* and in the case of massive colonisation could be treated with insecticide or destroyed to avoid pollen beetle dispersal.

The parasitism rate of *M. aeneus* larvae depended on plant species, parasitism rates were similar or higher on Brassica species than on *E. sativa, R. sativus* and *S. alba* (**I**, **II**). On most plant species the parasitism rate did not depend on host abundance. However, a moderate correlation was found between host abundance and parasitism rate on *R. sativus* (**I**, **II**). Hence, we conclude that the success of *M. aeneus*' parasitoid complex is not host density dependent. The parasitism rate varied from 20% up to 58% on *B. napus* between the years which was higher than previously reported from conventional fields in Estonia (0–16%) (Veromann *et al.*, 2006 a, d). The parasitism rate of *M. aeneus* exceeded 25% on all plant species except *E. sativa* and *R. sativus*. Parasitism rates were higher on

B. nigra (**I**) and *B. juncea* (**II**) than on *B. napus*. Parasitoids can effectively decrease populations of the pollen beetle when their parasitism level reaches 30–40% (Hokkanen, 2008), which was also exceeded on *B. rapa* and *S. alba* (**II**). Thus, we conclude that, on most studied plant species, parasitoids can effectively manage the abundance of *M. aeneus*.

Parasitoids identified during the study were *T. heterocerus, P. morionellus* and *D. capito*. These species commonly parasitise *M. aeneus* larvae throughout Europe (Osborne 1960; Nilsson & Andreasson 1987; Hokkanen, 1989; Nilsson 2003; Veromann *et al.* 2006a, b, d; Ulber *et al.*, 2010). In addition, one unidentified species was found rarely during the study (less than 1%). Surprisingly, one of the key species parasitizing *M. aeneus* throughout Europe, *P. interstitialis* (Nilsson, 2003), was not found although it regularly occurs in low numbers in Estonia (Veromann *et al.*, 2006a, d). It also has several advantages over other key species of parasitoids attacking *M. aeneus*, such as earlier arrival in the field and being more competitive than *T. heterocerus* in case of multiparasitism (Nitzsche & Ulber, 1998; Nilsson, 2003). *Phradis interstitialis* prefers to oviposit in *M. aeneus* eggs or first instar larvae (Nilsson, 2003), hence it is possible that due to earlier arrival the larvae parasitized by *P. interstitialis* had already dropped from plants to pupate. The lack of this species might also be related to its very low winter survival rate (Ferguson *et al.*, 2003).

The most abundant parasitoid species over the study was *T. heterocerus* although previous studies in northern Europe have found *Phradis* species to be predominant (Husberg & Hokkanen, 2001; Veromann *et al.*, 2006a, d). Veromann *et al.* (2006d) identified as many as 90% of parasitoids trapped in yellow water traps as *Phradis species.* The abundance of *T. heterocerus* was similar to that reported from central Europe where it is more important than *P. morionellus* and *D. capito* (Ulber *et al.*, 2010). Parasitisation by *D. capito* was more than three times higher than could be expected based on the abundance of adult parasitoids in northern Europe (Husberg & Hokkanen, 2001; Veromann *et al.*, 2006a, d). The high abundance of *M. aeneus* larvae parasitized by *D. capito* might be because of good overwintering conditions, usually the survival rate over the winter is very low (Nilsson, 2003). Also, this species might have high oviposition capability which could lead to high parasitism rate with low abundance of adults. To confirm this hypothesis additional studies are needed. Nevertheless, the percentage of *M. aeneus* larvae parasitized by

D. capito was 8%, while on *B. napus* and *S. alba* it has been reported in a range 8–29% (Billqvist & Ekbom, 2001).

Parasitoid species composition on different plant species is reported for the first time; it varied between plant species. On *B. napus* the abundance of different parasitoid species was relatively similar, and also in proportion with the overall species composition. *Phradis morionellus* was the most abundant on all *Brassica* species except on *B. nigra*. The most abundant parasitoid species over all plant species during the study was *T. heterocerus,* although no specimens were found from *E. sativa.* Its status was mainly achieved due to its high occurrence on *B. nigra*, where it constituted over 75% of all parasitoids. It differs from other Brassica species where the primary parasitoid species was *P. morionellus*. Also, contrary to *B. nigra* where one species significantly prevailed over others, on other Brassica species the abundance of the secondary parasitoid species varied from 18% to 33%.

The parasitoid species composition on *E. sativa, R. sativus* and *S. alba* differed from that on Brassica species as well as from each other. The predominant parasitoid species was *D. capito* on *R. sativus* and *P. morionellus* on *S. alba.* On *E. sativa D. capito* and *P. morionellus* were presented quite equally, but instead the most abundant parasitoid species over the study, *T. heterocerus* an unidentified species was present.

Phradis morionellus was the most efficient on *S. alba, B. rapa* and *B. juncea;* it oviposits in first and second instar larvae and is attracted by the scent of the bud (Nilsson & Andreasson, 1987; Jönsson *et al.*, 2005; Williams & Cook, 2010). As *P. morionellus* was more effective on plant species with lower host abundance, they are able to locate hosts on various cruciferous plants based on preferences of infested flowers. *Tersilochus heterocerus* gained its superiority on *B. nigra* which could be related to its host searching behaviour and host preferences. They are attracted by the odour of flowers and explore them despite host abundance to search for large second instar larvae in open flowers where they are more numerous (Winfield, 1963, Lehmann, 1965; Nilsson & Andreasson, 1987; Ferguson *et al.*, 2003; Jönsson *et al.*, 2005, Williams & Cook, 2010). Current results show their effectiveness during higher host abundance as the majority of them were found on the most infested plant species.

Thus, we conclude that, although *P. morionellus*, *D. capito* and *T. heterocerus* were present in similar numbers and equally represented on *B. napus*, their host finding success differs on other plant species. *Diospilus capito* is more successful on *E. sativa* and *R. sativus*. *Diospilus capito* showed the most efficient host locating skills as they were quite successful finding hosts from plants belonging to the Brassica family and also other plant families. *Brassica nigra* and *B. juncea* showed their potential as biological control-assisted trap crops, as the parasitism rate was higher on these plant species compared to that on *B. napus* and also different parasitoid species, *P. morionellus* and *T. heterocerus*, respectively, gained superiority. They also showed different searching behaviours, one favoured low and one high pest densities.

5.4. Potential plant species for dead-end trap cropping of *M. aeneus*

We showed the potential of *Raphanus sativus*, and to a certain degree also *E. sativa*, as dead-end trap crops for *M. aeneus* for the first time (**IV**). These were the only plant species in the current study where dead larvae were found.

The proportion of green and yellow buds infested by *M. aeneus* were similar on all *Brassica* species and also on *R. sativus*. *Sinapis alba* and *E. sativa* were less attractive, which is in concurrence with the findings of Ekbom (1998) who also observed the preference of *Brassica* species over *S. alba* and *E. sativa*. Although *M. aeneus* clutch size on *R. sativus* was somewhat lower than on *B. napus, B. nigra* and *B. rapa*, its mortality rate was greatest on this plant. A significant number of dead *M. aeneus* larvae was found also from *E. sativa*. Although several studies have found egg production and clutch size to be host plant quality dependent (Hopkins & Ekbom, 1996; Ekbom, 1998; Hopkins & Ekbom, 1999; Ekbom & Popov, 2004) it is likely that *M. aeneus* lacks capability to detect the suitability of *R. sativus*. Many larvae died on *R. sativus* during the moulting process, failing to shed their old cuticles; this is probably linked to a faulty hormonal balance. The moulting process is regulated by several hormones (Hiruma & Riddiford, 2001). Their balance in concurrence with other physiological changes is required for success (Nijhout, 1994; Davidowitz *et al.*, 2003). Inevitable changes in the endocrine system might be caused by low quality food from *R. sativus* or even by toxic compounds. As a 30% parasitism rate can effectively lower pollen beetle

populations (Hokkanen, 2008) we can assume that a similar mortality rate due to other reasons, such as poor host plant quality, may potentially have a similar impact.

The larvae on *E. sativa* died shortly after hatching. This could have been caused by low quality food or the presence of feeding deterrents. Several cruciferous plants contain compounds which act as feeding inhibitors for insects (Nielsen, 1989; Bartlet & Williams, 1991; Shinolda *et al.*, 2002; Metspalu *et al.*, 2013).

In conclusion, the equal attractiveness for oviposition in conjunction with its unsuitability for the development of pollen beetle larvae, *R. sativus* could be used as a dead end trap crop to protect *B. napus,* although additional studies are needed to establish its impact on other pests.

5.5. Manipulation with fertilisation

Nitrogen fertilisation had an impact on *M. aeneus* and also on its parasitoids in winter oilseed rape. While *M. aeneus* preferred plants with a low (60, 80 kg N ha^{-1}) or high (160 kg N ha^{-1}) fertilisation level, the highest parasitism rate was recorded on control plots. Although the colour yellow is attractive to *M. aeneus* (Giamoustaris & Mithen, 1996; Blight & Smart, 1999; Döring *et al.*, 2012; Cook *et al.*, 2013) and also for its parasitoids (Williams *et al.*, 2003; Johnen *et al.*, 2006; Williams & Cook, 2010) and the flower abundance increased continually with fertilisation, the abundance of insects was more complex. We conclude that other factors gained importance over the colour influencing both *M. aeneus* and its parasitoids. It is suggested that the scent of *B. napus* is the primary cue for both *M. aeneus* and its parasitoids for crop location; they are both attracted by *B. napus*' odour (Jönsson *et al.*, 2005, 2007; Williams *et al.*, 2007; Williams & Cook, 2010). Therefore, it can be assumed that olfactory cues had greater effect on insect behaviour.

Although nitrogen fertilisation can alter VOC emission of plants (Chen *et al.*, 2010), no clear separation among different nitrogen fertilisation levels were found during this study. However, the emission of several compounds increased with additional nitrogen. Especially the complex of green leaf volatiles and acetic acid had higher emission rates at higher fertilisation levels.

The emission rates of this compound were also positively correlated with larval abundance. *Meligethes aeneus* is more attracted to flowering plants than to the ones in bud stage (Cook *et al*., 2007b) and, in this study, several differences in VOC emission rates between bud and flowering stage were found and bouquets also separated clearly during the PCA analysis. As the most preferred size of bud for oviposition is 2–3 mm (Nilsson, 1988; Borg, 1996) volatiles emitted during this stage should be important in host location.

For instance, the emission rates of (3Z)-hexenyl acetate and camphene, which were positively correlated with the larval abundance of *M. aeneus*, were emitted at higher levels during the bud stage. On the other hand, the emission of acetic acid was negatively correlated with larval abundance and was emitted at lower levels during the bud stage and at lower fertilisation levels. Since the control plants were also less infested with beetle larvae, acetic acid may be one of the cues for females. Higher nitrogen amounts also increased the methyl salicylate emission, this compound has been recently reported to attract beneficial insects including parasitoids (Rodriguez-Saona *et al*., 2011; Kaplan, 2012).

Parasitoids also use volatile cues provided by plants as often these are more reliable and also due to the higher biomass of plant compared to herbivorous insects, can be detected from farther away. Parasitoids of *Meligethes aeneus* are attracted to volatiles emitted during the bud stage and for some species the attractiveness can be increased when combined with yellow colour (Jönsson, 2005). *Phradis morionellus* is also known to respond to olfactory cues, from the VOCs recorded during this study they are reported to respond to (E,E)-α-farnesene, benzaldehyde and indole.

CONCLUSIONS

1. The abundance of *M. aeneus* and its parasitoids could be manipulated by the selection of plant species (**I**, **II**, **III**, **IV**) and nitrogen fertilisation levels (**V**).

2. The emission of VOC-s of *B. napus* varied with growth stage and nitrogen fertilisation levels (**V**). *Meligethes aeneus* abundance could be manipulated with several VOC-s, positively with (3Z)-hexenyl acetate, camphene, 3-carene, limonene, indole, acetic acid and negatively with β-pinene, linalool, (E,E)-α-farnesene, benzaldehyde, methylbenzoate.

3. The selection of plant species for trap-cropping for *M. aeneus* depended on the utilization purpose. For conventional trap cropping: *S. alba* and *B. nigra* to avoid *M. aeneus* feeding damage during the green-yellow bud stage (**II**, **III**) and *B. nigra, R. sativus* to attract *M. aeneus* for oviposition (**III**, **IV**). *Raphanus sativus* had potential to be used as a dead end trap crop for *M. aeneus*. *Raphanus sativus* and *B. napus* were equally attractive to *M. aeneus* for oviposition but the former did not support the development of the larvae (**IV**).

4. Host plant species affected the host finding success of *M. aeneus* parasitoids. On most plant species (except *R. sativus*) parasitism rate of *M. aeneus* was not host abundance dependent. *Brassica juncea* and *B. nigra* had suitable properties to be used as biological control-assisted trap crops for *M. aeneus* due to the high parasitism rate (**I**, **II**).

5. *Meligethes aeneus* parasitism rate and parasitoids' species composition was influenced by plant species. *Brassica juncea*, *B. nigra*, *B. rapa*, *E. sativa*, *R. sativus* and *S. alba* provided suitable habitat for pollen beetle parasitoid species not predominant on *B. napus* (**I**, **II**).

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SUMMARY

The importance and hence growing area of oilseed rape is likely to increase in the future. But the current pest control methods have negative environmental implications and are also losing their efficiency.

This study investigated 1) the attractiveness and impact plant species and nitrogen fertilisation have on the feeding and oviposition preferences of *M. aeneus* and its parasitoids and 2) the impact of nitrogen fertilisation on volatile organic compounds' emission. The aim was to gain knowledge on how to manipulate *M. aeneus'* abundance through their preferences.

The feeding and oviposition preferences of *M. aeneus* and its parasitoids was studied on *Brassica napus*, *Sinapis alba*, *Eruca sativa*, *Raphanus sativus*, *B. nigra*, *B. rapa* and *B. juncea* (Capparales: Brassicaceae). The results showed the dependence of plant species and growth stages on *M. aeneus*' feeding and oviposition preferences. During the green-yellow bud stage (BBCH 50–59) *S. alba* and *B. nigra* were the most attractive plant species. This is the most damage-susceptible phase of *B. napus*, showing these plants' potential as trap-crops for *M. aeneus* to prevent oilseed rape fields colonisation by overwintered adults.

The plant species also influenced the number of buds used for oviposition by *M. aeneus* and clutch size. For oviposition, species of the genus Brassica was preferred over *S. alba* and *E. sativa* but not over *R. sativus*. The clutch size was smaller also on *R. sativus* compared to Brassica species. The assessment of larval abundance showed higher numbers generally on *B. napus* but depending on year the attractiveness of *B. nigra* was similar or even higher. These results show probabilities to use different plant species to manipulate with *M. aeneus* oviposition preferences, to collect larvae to certain areas on the field where the trap crop can be destroyed in case of high abundance.

This raised the need to investigate whether possible dead-end trap crop (attractive for oviposition without suitable features for larval development) for *M. aeneus* also exists. Larval survival rate was studied on a variety of Brassicaceous plant species but dead larvae were found only on *R. sativus* and *E. sativa*. *Eruca sativa* vas not very attractive for oviposition. But *R. sativus* was as attractive as *B. napus* and one third of larvae failed to

survive. Their death occurred before or during moulting to second instar suggesting unsuitable food composition or lack of required nutrients. From investigated plant species, *R. sativus* showed the most potential to be used as dead-end trap crop for *M. aeneus*.

The influence of plant species was also confirmed for third tropic level, parasitoids. *Brassic juncea* and *B. nigra* had a higher percentage of parasitized larvae than *B. napus*, which shows their potential for biological control-assisted trap crop. Nevertheless, as the parasitism rate from 30% can significantly lower the pests' abundance, also *B. rapa* and *S. alba* support the abundance and distribution of parasitoids. Four species of parasitoid were found: *Tersilochus heterocerus* (43%), *Phradis morionellus* (32%), *Diospilus capito* (25%) and one unidentified species with low occurrence (<1%). From all plant species but *E. sativa* all major species of parasitoids were found but their species composition differed. On most Brassica species *P. morionellus* gain superiority, but on *B. nigra T. heterocerus* was the most abundant species. *Diospilus capito* was predominant species on *R. sativus.* Therefore parasitoids can be influenced with plant species selection to support the species which are not prevalent on *B. napus.*

Fertilisation also had impact on both pest and parasitoids, probably via VOC emission, which also differed with N fertilisation levels. *Meligethes aeneus* preferred to lay eggs on moderate and high nitrogen levels while parasitism rate was the highest on zero fertilisation level. Analysis showed the highest emission rates for acetic acid, 3-carene, α-pinene and benzaldehyde, the emission rates of several VOCs increased with nitrogen fertilisation. The abundance of *M. aeneus* larvae were positively correlated with emission of (3Z)-hexenyl acetate, camphene, 3-carene, limonene, indole, acetic acid and negatively with β-pinene, linalool, (E,E)-α-farnesene, benzaldehyde, methylbenzoate emission rates. Several parasitoid species are attracted by volatiles emitted by plants their hosts feed on. *Phradis morionellus* is also known to respond to olfactory cues, from the VOC's recorded during current study they are reported to respond to (E,E)-α-farnesene, benzaldehyde and indole.

The results of this study show the potential for use of different plant species on the management of *M. aeneus* and its parasitoids. In conditions similar to Estonia *B. nigra* and *S. alba* have potential for conventional trap cropping to prevent damage caused by overwintered *M. aeneus* adults. *Brassica nigra* was also attractive for oviposition which can be used

to influence the forthcoming generation. For dead-end trap cropping, the most promising plant species was *R. sativus,* the biological control-assisted trap cropping on *B. napus* can be promoted by using *B. nigra* and *B. juncea*. By increasing the abundance and species diversity of parasitoids the damage caused by *M. aeneus* could be kept below economic threshold levels. Such precautionary methods can be used in integrated and organic farming.

Pest and parasitoid abundance can also be manipulated by optimising nitrogen fertilisation. By using moderate nitrogen input the pressure on environment can be reduced.

SUMMARY IN ESTONIAN

Tritroofsete suhete rakendamine jätkusuutliku taimekaitse strateegia leidmiseks rapsile

Raps (*Brassica napus* L.) on üks tähtsamaid parasvöötmes kasvatatavaid ristõielisi kultuure ja üks tähtsaimaid õlikultuure Euroopas. Laialdane kasvatamine ilma piisava ruumilise ja ajalise eraldatuseta on viinud laialdase kahjurite levikuni.

Üheks olulisemaks kahjuriks nii tali- kui suvirapsil on kogu Euroopas naeri-hiilamardikas (*Meligethes aeneus* F., Coleoptera: Nitidulidae). Siiani on selle kahjuri arvukuse reguleerimiseks kasutatud sünteetilisi taimekaitsevahendeid, peamiselt püretroide. Sageli on tõrjeskeemid baseerunud tehnoloogilisele skeemile ja taimekaitsevahendeid on kasutatud rutiinselt ning profülaktiliselt majanduslikke tõrjekriteeriume jälgimata. Pestitsiidide laialdase ja rutiinse kasutamise tagajärjel on hiilamardikatel väljakujunenud resistentsus püretroidide suhtes kogu Euroopas. Lisaks sellele kaasneb ohtra pestitsiidide kasutamisega saagi majanduslik konkurentsivõime langus ja suureneb keskkonna saastamise oht, samuti hävitatakse kasulikke ja mitte-sihtgrupi organisme. Samas saaksid kasulikud organismid põllumehele otsest kasu tuua kahjurite hävitamise ja tolmeldamise näol. Seetõttu on vajalik arendada jätkusuutlikumaid lahendusi kahjurite haldamiseks.

Üheks keskkonnasäästlikuks lahenduseks rapsikasvatuses on püüniskultuuri-strateegia, mis manipuleerib kahjuritega kasutades nende toitumis- ja munemiseelistusi. Antud taimekaitsevõte põhineb erinevate kahjurile atraktiivsemate taimeliikide või sortide kasutamisel põhikultuuri kaitseks ja/või looduslike vaenlaste soodustamiseks. Samas on sellise strateegia rakendamiseks vajalikud süvateadmised kahjuri eluringi, toidu- ja munemiseelistuste, talvitumise, looduslike vaenlaste jne kohta.

Käesoleva töö eesmärgiks oligi välja selgitada erinevate taimeliikide potentsiaal eri suunitlustega püüniskultuuri-strateegia arendamiseks suvirapsile: i) millised on naeri-hiilamardika munemis- ja toitumiseelistused erinevatel taimeliikidel (**I**, **II**, **III**, **IV**); ii) kas ja kuidas mõjutavad erinevad taimeliigid parasitoidide otsingukäitumist peremehe leidmisel ja nende liigilist koosseisu (**I**, **II**); iii) millised on potentsiaalsed taimeliigid naeri-hiila-

mardikale surmava püüniskultuuri (dead-end trap crop) leidmiseks (**IV**); iv) kas ja kuidas mõjutab lämmastikuga väetamine naeri-hiilamardika ja tema parasitoide arvukust ning kas sellel on seos lenduvate orgaaniliste ühendite eritamisega taimelt (**V**).

Katsetööd erinevate ristõieliste kultuuridega viidi läbi aastatel 2006–2012 Eesti Maaülikooli katsemaadel ja talirapsi väetuskatsed seitsme erineva lämmastikunormiga (0 kuni 160 kg N ha^{-1}) viidi läbi aastatel 2008–2009 Jõgeva Sordiaretuse Instituudis.

Naeri-hiilamardika toitumis- ja munemiseelistusi uuriti seitsmel erineval ristõielisel taimeliigil: raps (*B. napus)*, rüps (*B. rapa*), must sinep (*B. nigra*), kapsasrohi (*B. juncea*) perekonnast kapsasrohi; valge sinep (*Sinapis alba)* perekonnast sinep; põld-võõrkapsas (*Eruca sativa*) perekonnast võõrkapsas ja õlirõigas (*Raphanus sativus*) perekonnast rõigas (Capparales: Brassicaceae).

Kahjuri arvukuse hindamiseks loendati valmikud, vastsed ja munad eri taimeliikidel; parasiteerituse hindamiseks ja parasitoidide liigilise koosseisu väljaselgitamiseks lahati teise kasvujärgu vastsed; naeri-hiilamardika suremuse hindamiseks eri taimeliikidel loendati taimede roheliste ja kollaste pungade kasvustaadiumites (BBCH 51–59) munade, vastsete ja surnud vastete hulk.

Kahjuri toitumis- ja munemis-eelistusete uurimisel selgus, et kõik taimeliigid sobisid naeri-hiilamardikale toitumiseks ja munemiseks, kuid nende atraktiivsus oli erinev nii taime liikide kui ka kasvufaaside vahel. Rohelise-kollase punga faasis, mil raps on kõige haavatavam naeri-hiilamardika kahjustusele olid mardikatele rapsist oluliselt atraktiivsemateks taimeliikideks valge ja must sinep (**II**, **III**). Kuna alates õitsemise faasist on naeri-hiilamardika valmikute ja ka vastsete poolt tekitatav kahju tühine ning majanduslikult olulist saagilangust ei põhjusta, siis on meie tulemuste põhjal järeldada, et nendel taimeliikidel on potentsiaali traditsioonilise püüniskultuurina. Musta- ja valget sinepit püüniskultuurina kasutades saaks vähendada talvitumast tulnud naeri-hiilamardikate valmikute invasiooni rapsipõllule enne kultuuri õitsema hakkamist (**II**, **III**).

Kuigi naeri-hiilamardika vastsete toitumine lahtistel õitel ei põhjusta majanduslikult olulist saagilangust, tekitavad kinnistes õites toituvad vastsed siiski kahjustusi. Nende toitumise tagajärjel võivad kahjustada saada

õite sigimikud, mille tulemusel ei moodustu kõtrasid ja taimedele jäävad vaid kõtrade varred (blind stalks). Samas vallandab see ka taimepoolse kompenseerimismehhanismi, mille käigus toimub uute õiepungade kasvatamine. Reproduktiivorganite kasvatamine on aga taimele „kulukas" ja nõuab lisaressursse toitainete näol. Selleks uuriti naeri-hiilamardika munemis-eelistusi erinevatel ristõielistel taimeliikidel rohelise ja kollase punga ning õitsemise staadiumites (**I**, **II**, **III**, **IV**). Tulemused näitasid kapsasrohu perekonda kuuluvate liikide eelistamist valgele sinepile ja põld-võõrkapsale, kuid mitte õlirõikale. Samas oli munakurna suurus ka õlirõikal mõnevõrra väiksem kapsasrohu perekonda kuuluvate liikide omadest (**IV**). Lisaks selgus, et taimeliigil on oluline mõju nii kasutatud pungade arvule kui ka kurna suurusele (**IV**). Vastsete arvukuse hindamisel selgus, et enamasti on vastsed arvukaimad rapsil (**II**), kuid olenevalt aastast on ka must sinep atraktiivsuselt vähemalt samaväärne (**I**) või isegi atraktiivsem (**III**).

Antud tulemused näitasid võimalust mõjutada naeri-hiilamardika munemiseelistusi. Kasutades atraktiivseid taimeliike, saaks kontsentreerida vastsete paiknemist põllu servaaladele ja siis vastsete tiheda asustuse korral püüniskultuur hävitada. Sellest lähtuvalt kerkis vajadus uurida, kas ka rapsile on võimalik leida surmavat püüniskultuuri, mis oleks naeri-hiilamardikale väga meelitav munemispaik, kuid ei toetaks vastsete arengut. Selleks hinnati erinevatel taimeliikidel elusate ja hukkunud vastsete hulk (**IV**). Vastsete hukkumist täheldati vaid õlirõikal ja põld-võõrkapsal, millest viimane oli aga munemiseks väheatraktiivne. Õlirõikal ei suutnud ligi kolmandik vastsetest arengut edukalt lõpule viia. Enamasti toimus hukkumine vahetult enne teise kasvujärku kestumist või selle käigus, mis viitab toiduallika ebasobivale koostisele või vajalike toitainete puudumisele. Õlirõika atraktiivsus munemispaigana ja rohke vastsete hukkumine näitab, et antud taimeliigil on potentsiaali, et teda rakendada surmava püüniskultuurina vähendamaks naeri-hiilamardika populatsiooni.

Parasiteerituse hindamiseks ja parasitoidide liigilise koosseisu väljaselgitamiseks lahati 4666 naeri-hiilamardika vastset, millest kokku avastati 1430 parasitoidi muna või vastet (**I**, **II**). Antud katsetest selgus, et taimeliigil on oluline mõju nii parasiteerituse taseme suurusele kui ka parasitoidide liigilisele koosseisule. Naeri-hiilamardika parasiteeritus oli rapsiga võrreldes samaväärne või kõrgem kõigil taimeliikidel peale põld-võõrkapsa ja õlirõika. Bioloogilist kontrolli toetava püüniskultuuristrateegia arendamiseks sobilikke omadusi näitasid must sinep ja kapsasrohi. Samas

põhinedes varasematele uuringutele, mis näitasid et parasiteerituse määr juba alates 30%-st vähendab oluliselt kahjuri arvukust, võib järeldada, et ka rüps ja valge sinep toetavad parasitoidide arvukust ja levikut.

Uurimustöö käigus leiti parasitoide neljast erinevast liigist (**I**, **II**), kellest kolm: *Tersilochus heterocerus, Phradis morionellus* ja *Diospilus capito* on võtmetähtsusega liigid naeri-hiilamardika arvukuse reguleerimisel kogu Euroopas. Neljas liik, keda ei õnnestunud määrata moodustas <1% kogu parasitoide hulgast. Parasitoididest 43% moodustas *T. heterocerus* (608 isendit), 32% *P. morionellus* ja 25% *D. capito*.

Lisaks parasiteerituse tasemele selgus, et taimeliikidel on oluline mõju ka parasitoidide liigilisele koosseisule. Kapsasrohu perekonda kuuluvatel liikidel, va must sinep, oli peamiste parasitoidide osakaal suhteliselt võrdne ning sarnane: enim naeri-hiilamardika vastsetest oli parasiteeritud *P. morionellus*-e poolt, kuid teiste liikide suhteline tähtsus varieerus vaid 18–23 %. Seevastu valgel sinepil oli parasitoidi *P. morionellus* osakaal üle 70% ja vaid 21% oli parasiteeritud katse arvukaimalt esindatud parasitoidi *T. heterocerus*-e poolt. Samas oli mustal sinepil ülekaalukalt arvukamalt esindatud liik just *T. heterocerus*, mis moodustas tervelt 75% isenditest, liikide *D. capito* ja *P. morionellus* esindatus oli suhteliselt võrdväärne.

Õlirõikal oli suurima tähtsusega liik *D. capito* ja Euroopas enimlevinud parasitoid *T. heterocerus* panustas sellel taimeliigil naeri-hiilamardika kontrolli vaid 7%. Põld-võõrkapsal oli samuti arvukaimaks parasitoidiks *D. capito* (50%), kellele järgnes *P. morionellus*; huvitaval kombel ei leitud sellel taimel ühtegi *T. heterocerus* poolt parasiteeritud naeri-hiilamardika vastset, 17% parasitoididest moodustas hoopis määramatu liik.

Enamik taimeliike pakkus sobivat arengukeskkonda kõigile peamiste parasitoididele, kuid erinevates proportsioonides. Seega saab parasitoidide liiglist koosseisu ja mitmekesisust mõjutada tema peremehe toidutaime valikuga ja vastavalt sellele soodustada ka neid liiki parasitoide, mis ei domineeri peamiselt rapsil.

Talirapsil läbiviidud väetamiskatsete tulemused näitasid, et lämmastikuga väetamine mõjutab nii naeri-hiilamardika kui ka tema parasitoidide arvukust (**V**). Kõige vähem vastseid leiti katsevariandilt, kus lämmastikku ei lisatud, mistõttu seal oli ka taimede vegetatiivne kasv nõrgem. Vastsete

arvukus oli suurim madala (60–80 kg ha^{-1}) ja kõrge (160 kg ha^{-1}) väetustaseme juures. Parasitoididele oli seevastu atraktiivseim just katsevariant, kuhu lämmastikku ei lisatud, kuid positiivne seos leiti vastsete ja parasiteeritud vastete arvu vahel.

Lenduvate ühendite analüüsimisel leiti 19 erinevat ühendit, raps eritas enim 3-kareeni, α-pineeni ja bensoealdehüüdi. Kõrgemate lämmastikväetise koguste juures suurenes ka mitmete ainete emiteerimine, esmajoones 1-isopropüül-4-metüül tsüklohekseeni, äädikhappe ja LOX-i (taimede rohelistest osadest lenduvate ühendite kompleks) hulk. Kuid nii punga kui õitsemise faasis suurenes lämmastiku lisamisel vaid kahe ühendi: metüül-2-hüdroksübensoaat ja äädikhappe eritumine.

Mitmete lenduvate ühendite eritumine oli kas positiivses või negatiivses korrelatsioonis naeri-hiilamardika vastsete arvuga taimel, kõrgem 3-kareeni, indooli ja limoneeni eritamisel oli vastsete arvukus taime kohta suurem, samas kui β-pineiini, (E,E)-α-farneseeni, metüülbensoaadi, bensoealdehüüdi ja linalooli kõrgem eritamine mõjus naeri-hiilamardika munemisaktiivsusele pärssivalt. Et parasiteeritud vastsete arv korreleerus vastsete koguarvuga võib järeldada, et nad kas jagasid oma potentsiaali võrdselt kõigil katsevariantidel või lähtuvad sarnasest keemilistest vihjetest. Siiski on teada, et parasitoidid reageerivad mitmetele rapsi lenduvatele ühenditele, eelnimetatud ühenditest vähemalt kolmele ((E,E)-α-farneseen, indool ja bensoealdehüüd) reageerib positiivselt ka naeri-hiilamardika parasitoid *P. morionellus*.

Kokkuvõtteks saame järeldada, et naeri-hiilamardika arvukuse kontrollimiseks saab suvirapsil rakendada mitmeid keskkonnasõbralikke põllumajanduslikke võtteid. Eesti tingimustes on potentsiaalsed traditsioonilised püüniskultuurid naeri-hiilamardika valmikutele must ja valge sinep. Lisaks sellele oli must sinep ka atraktiivne munemistaimena, mida saab ära kasutada hiilamardika uue põlvkonna arvukusega manipuleerimise eesmärgil. Meelitades putukad püüniskultuuridele saab viimased hävitada kas insektitsiide kasutades või siis püüniskultuuri sisse kündes. Viimast strateegiat saaks soovitada ka mahepõllumajanduses kasutamiseks. Surmava püüniskultuuri strateegia arendamiseks oli kõige sobivam kultuur õlirõigas.

Samuti saab erievate taimeliikide abil toetada naeri-hiilamardika parasitoidide arvukust ja nende liigilist koosseisu. Mustal sinepil ja kapsasrohul

ning teatud määral ka rüpsil ja valgel sinepil on potentsiaali bioloogilist kontrolli toetava püüniskultuuristrateegia arendamiseks. Suurendades parasitoidide arvukust ja ka mitmekesistades nende liigilist koosseisu rapsipõldudel saame hoida hiilamardika populatsiooni suuruse looduslikul teel tõrjekriteeriumist madalama. Sellisel ennetava tõrje võttel baseerub kahjurite regulatsioon nii integreeritud kui ka mahetootmises.

Lisaks on võimalik nii kahjureid kui ka nende looduslikke vaenlasi mõjutada optimeerides lämmastikuga väetamise taset. Kasutades keskmist väetamisetaset saame vähendada koormust keskkonnale ja koos sellega muuta kultuuri kahjurile ka vähem atraktiivseks.

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I

PUBLICATIONS

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Meligethes aeneus oviposition preferences, larval parasitism rate and species composition of parasitoids on *Brassica nigra*, *Raphanus sativus* and *Eruca sativa* compared with on *Brassica napus*

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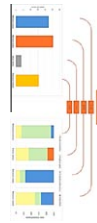
HIGHLIGHTS

- Oviposition rate of the pollen beetle varies with plant species.
- The species composition of pollen beetles' parasitoids varies with plant species.
- *Brassica nigra* has a potential to reinforce the natural control of the pollen beetle.
- *Brassica nigra* could be used as a parasitoid bank.

GRAPHICAL ABSTRACT



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ABSTRACT

The trap crop strategy is based on host plant discrimination by pests and their parasitoids, which may respond differently to various host plant cues, thus affecting their respective population distributions. We conducted a three-year study to compare the responses of the most damaging pest of oilseed rape (*Brassica napus* L.), the pollen beetle (*Meligethes aeneus* Fab.), and its hymenopteran parasitoids to various potential trap crops: *Brassica nigra* L., *Raphanus sativus* var. *olifera* Pers. and *Eruca sativa* Mill. with that to *B. napus*. We recorded their abundance, oviposition preferences and the species composition of the parasitoids.

Our results show that oviposition rates of the pollen beetle and its parasitoids as well the species composition of the parasitoids varies with plant species. We discuss the potential of these plant species, especially *B. nigra*, to enhance the natural control of the beetle by fostering several parasitoid species. The species composition of the parasitoids on different host plants compared with on *B. napus* is presented for the first time. In addition to trapping pests, the trap crops could also act as parasitoid banks, enhancing natural control of the pest through providing suitable hosts for natural enemies, without increasing the population growth of the next generation of pests.

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1. Introduction

One of the most important principles of integrated pest management (IPM) is the prevention and/or suppression of harmful organisms, encouraging the use of non-chemical methods and target-specificity to control pest abundance (BiPRO, 2009). One of the tools that addresses these principles is the use of a trap crop strategy for the pests and their naturally-occurring biocontrol agents i.e. the natural enemies of pests, including parasitoids.

As phytophagous insects locate the crop by responding behaviorally to different visual and olfactory cues, manipulation of these cues and hence of pest behavior can be used to reduce or avoid pest damage to the crop. Trap cropping aims to reduce pest colonization in the main crop by attracting pests to areas of trap crop planted close to the main crop (Hokkanen, 1991; Cook et al., 2007a,b, 2013). If the plant species used as a trap crop is also attractive to parasitoids and the percentage of parasitism is high enough to control the population size of their host insect, there is no need to destroy the trap crop or to treat it with insecticides. Under these circumstances, the trap crop can also perform as a parasitoid bank and support the increase of diversity and abundance of beneficial arthropods.

Oilseed rape (*Brassica napus* spp. *oleifera* L.) (Brassicaceae) is the third most widely grown crop in the European Union (FAO, 2013). In Estonia, the area grown has increased 82-fold over the past 20 years reaching 86 700 hectares in 2012 (Statistics Estonia, 2013). This increase has supported the population growth of crucifer-specialist pests. One of the most damaging pest throughout Europe is the pollen beetle (*Meligethes aeneus* (Fabricius) (Coleoptera: Nitidulidae)) (Alford et al., 2003; Cook and Denholm, 2008; Ekbom, 2010; Veromann et al., 2006a,b,c, 2008; Williams, 2010). Adult pollen beetles feed on pollen from plants belonging to different families (Free and Williams, 1978; Fritzsche, 1957; Williams, 2010), but oviposit only in buds of brassicaceous plants (Ekbom and Borg, 1996; Free and Williams, 1978; Nilsson, 1989) although they have behavioral preferences for some *Brassica* species over others (Buechi, 1990; Ekbom and Borg, 1996).

Generally, the abundance of pollen beetles is controlled by applying synthetic insecticides (Thieme et al., 2010), which may not solve the pest problem (Hokkanen, 2000) and can even increase it (Veromann et al., 2008). Another problem is the development of pyrethroid resistance in pollen beetles (Hansen, 2003, 2008; Heimbach et al., 2006; Cook and Denholm, 2008; Thieme et al., 2010; Tiilikainen and Hokkanen, 2008). Further, pesticides have a detrimental effect on parasitoids that are essential enemies of many crop pests and may act as keystone species in ecosystems (Murchie et al., 1997; Thies et al., 2003; Veromann et al., 2011).

In Europe, the key species of parasitoids controlling the abundance of pollen beetle are *Phradis interstitialis* Thomson, *Phradis morionellus* Holmgren, *Tersilochus heterocerus* Thomson (Hymenoptera: Ichneumonidae) and *Diospilus capito* Nees (Hymenoptera: Braconidae) (Nilsson, 2003). Of these, adults of *T. heterocerus* and *P. morionellus* commonly colonize the crop at the same time – at the beginning of flowering of either spring or winter oilseed rape varieties (Ulber and Nitzsche, 2006; Ulber et al., 2010; Williams, 2006) while *D. capito* is a multivoltine species which gains more importance when spring varieties start to flower (Miczulski, 1967; Nilsson, 2003). *Tersilochus heterocerus*, *P. morionellus* and *D. capito* oviposit into small larvae within buds and large second instar larvae in open flowers (Börner and Blunck, 1920; Nilsson, 2003; Osborne, 1960). *Diospilus capito* is mainly distributed in northern Europe and more common on spring oilseed rape (Hokkanen, 2008; Nilsson, 2003; Veromann et al., 2006a,b).

The average parasitism percentage of pollen beetle larvae varies between 25% and 50% in Europe (Ulber et al., 2010), but can reach 90% (Ulber et al., 2006). The abundance of this pest can be effectively lowered with a parasitism rate of 30–40% (Hokkanen, 2008). In Estonia, a parasitism rate of 48% has been reported (Veromann et al., 2013), although in conventional cropping systems it is more usually under 4% (Veromann et al., 2009).

The potential of trap cropping to reduce insecticide treatments and to avoid damage caused by pollen beetles in oilseed rape has been intensively studied (Buechi, 1990; Cook et al., 2006; Cook and Denholm, 2008; Ekbom and Borg, 1996; Veromann et al., 2012) as has the potential of parasitoids to control the pest (Ekbom, 2010; Ferguson et al., 2003; Hokkanen, 1991, 1989, 2006; Jönsson et al., 2004; Kromp and Kraus, 2006; Nilsson, 2003; Nilsson and Ahman, 2006; Nilsson and Andreasson, 1987; Nitzsche and Ulber, 1998; Osborne, 1960; Ulber et al., 2010). However, potential trap crops other than *B. rapa* have received only minor attention so far (Hokkanen et al., 1986; Kovács et al., 2013; Veromann et al., 2012) and the potential of parasitoids has only been investigated separately on plant species other than *B. napus* (Billqvist and Ekbom, 2001a,b). The effects of potential trap crops on the efficiency and species composition of parasitoids remain unexplored.

In this study, we hypothesized that cruciferous plants differ in their attractiveness for oviposition to pollen beetle adults and their larval parasitoids. To test this hypothesis, we compared the oviposition preferences of the pollen beetle and its parasitoids for the potential cruciferous host plants *Brassica nigra* (L.) W. D. J. Koch (syn. *Sinapis nigra* L.), *Raphanus sativus* L. var. *oleiformis* Pers. and *Eruca sativa* Mill. (syn. *Eruca vesicaria* (L.) Cav.) with that for spring oilseed rape.

2. Materials and methods

2.1. Study area and experimental design

Studies were carried out in an experimental field of the Estonian University of Life Sciences, Tartu, between summers 2009 and 2011. The experiment was laid out in a randomized complete block design with three replicates of each plant species: *B. napus*, *R. sativus*, *B. nigra* and *E. sativa*. Each plot was 1 × 5 m with 1 m wide bare soil buffer zone around each plot. Neither fertilizers nor pesticides were applied.

2.2. Plant material

Plots were sown on 7 May 2009, 12 May 2010 and 9 May 2011 at 250 seeds per m^2. In 2009, seeds of *B. nigra* and *E. sativa* cv. 'Poker' of Gavris Plant-breeding and Seed-production Company were purchased from Hansaplant LLC seed company and seeds of *B. napus* spring variety cv. '*Maskot*' of Svalöf Weibull AB (the company's current name is Lantmännen SW Seed AB) and *R. sativus* cv.'*Bille*' of GSS Saatzucht Salzmünde GmbH, *E. sativa* were obtained from the seed collection of the Estonian University of Life

Table 1
The sampling dates of *M. aeneus* larvae from different plants at growth stage 64–67 (BBCH, Lancashire et al., 1991) on four cruciferous plant species tested 2009–2011.

	2009	2010	2011
Brassica napus	7.07	5.07	11.07
Brassica nigra	7.07	29.06	7.07
Eruca sativa	14.07	9.07	11.07
Raphanus sativus	14.07	12.07	11.07

Sciences. The cultivar of *B. napus* used in this study is early to medium maturing with medium height. The *R. sativus* cultivar has pale violet flowers. In 2010 and 2011, seeds from the previous year's study were sown.

2.3. Insect sampling

To estimate pollen beetle oviposition activity, the buds and flowers of ten randomly chosen plants were collected from each plot at full flowering stage (GS 64–67; Lancashire et al. 1991; Table 1), dissected in the laboratory and all larvae counted.

To determine the parasitisation rate of the pollen beetle larvae, second instar larvae i.e. the last larval stage before dropping to the ground to pupate in the soil, were dissected under a stereoscopic microscope (Olympus SZ-CTV). Different numbers of specimens were dissected depending on plant species: 912 from *B. napus*, 1140 from *B. nigra*, 140 from *E. sativa* and 599 from *R. sativus*. Parasitoid larvae and eggs were counted and the percentage parasitism was calculated. Parasitoid larvae and eggs were identified to species using the key by Osborne (1960).

2.4. Statistical analyses

To test the statistical significance of plant species and year on the flower supply and on the number of beetle larvae per plant, the Poisson model considering fixed effects of year and plant species and random effect of replicate was applied (GLIMMIX Procedure). In parasitism rate analyses the logistic model with the same factors was used. The same models only without year effect were used to compare the flower supply, number of beetle larvae per plant and parasitism rate between plant species for each year. The modeling results are presented as least square means (± standard error). The species composition of the larval endoparasitoids of the beetle was compared with the Fisher exact test. Spearman correlation analysis was used to study the relationship between flower supply, number of beetle larvae and their parasitism rate. The analyses were conducted with SAS 9.1 software (SAS Institute, Inc., Cary, NC, USA). All results were considered statistically significant at $P < 0.05$.

3. Results

Plant species had a significant influence on the abundance of pollen beetle larvae over the three-year study period ($F = 14.07$, df = 3, $P < 0.0001$) but year as a factor had no influence ($F = 2.93$, df = 2, $P = 0.069$). The effect of plant species was also significant each year (2009: $F = 33.07$, df = 3, $P < 0.0001$; 2010: $F = 16.52$, df = 3, $P = 0.0009$; 2011: $F = 9.09$, df = 3, $P = 0.0059$). For oviposition in 2009, *B. nigra* and *B. napus* were preferred over *E. sativa* and *R. sativus* ($P \leqslant 0.001$; Fig. 1); in 2010, the greatest number of beetle larvae per plant was found from *B. napus*, more than from *B. nigra* and *E. sativa* ($P < 0.05$); *E. sativa*, which had the lowest infestation rate, differed also from *B. nigra* and *R. sativus* ($P < 0.001$). In 2011, the abundance of beetle larvae was similar on *B. napus*, *B. nigra* and *R. sativus*, with significantly fewer on *E. sativa* ($P < 0.01$).

The number of flowers per plant varied with plant species ($F = 9.35$, df = 3, $P = 0.0002$), with significantly more on *B. nigra* ($t = -2.92$, df = 30, $P = 0.0065$) and fewer on *E. sativa* ($t = 2.32$, df = 30, $P = 0.027$; Fig. 2) than on *B. napus*. The number of flowers per plant also differed with year ($F = 9.35$, df = 3, $P = 0.0002$).

The parasitism rates of beetle larvae differed with plant species ($F = 23.54$, df = 3, $P < 0.0001$) but not year ($F = 1.77$, df = 2, $P = 0.19$). Biological control by parasitoids was the most efficient on Brassica species, especially on *B. nigra*, where the parasitism rate was higher than on all other plant species ($P < 0.05$) and a similar tendency was followed each year, even though no significant differences were found (Fig. 3).

Flower supply affected the abundance of beetle larvae only weakly (*B. nigra*: $r = 0.22$, $N = 90$, $P = 0.037$; *B. napus*: $r = -0.15$, $N = 90$, $P = 0.14$; *E. sativa*: $r = -0.33$, $N = 90$, $P = 0.0017$ and *R. sativus*: $r = 0.049$, $N = 90$, $P = 0.65$); also no statistical support was found to indicate that the parasitism rate depends on host abundance on most plant species (*B. nigra*: $r = 0.014$, $N = 75$, $P = 0.90$), *B. napus*: $r = 0.067$, $N = 84$, $P = 0.54$; *E.* sativa: $r = 0.19$, $N = 46$, $P = 0.20$); however, on *R. sativus* this relationship was moderate ($r = 0.37$, $N = 51$, $P = 0.0083$). In addition, no significant correlations between parasitism rate and flower supply were found (*B. nigra*: $r = -0.047$, $N = 75$, $P = 0.69$), *B. napus*: $r = 0.11$, $N = 84$, $P = 0.32$; *E.* sativa: $r = -0.23$, $N = 46$, $P = 0.13$; *R. sativus*: $r = -0.18$, $N = 51$, $P = 0.20$) on all studied plant species.

In total, 805 hymenopteran parasitoids of four species: *T. heterocerus*, *P. morionellus*, *D. capito* and one unidentified species, named the "healthy-fat-one", were detected from beetle larvae during the three year study (Fig. 3). The most common species was *T. heterocerus*, comprising over 50% of all parasitoids found during the study. Nearly one quarter of parasitoids was identified as *P. morionellus* and 17% as *D. capito*. In total, six parasitoid larvae of the "healthy-fat-one" type remained unidentified but over the study they formed less than 1% of all parasitoids.

Species composition of parasitoids differed significantly with plant species ($\chi^2 = 305.97$, df = 9, $P < 0.0001$; Fig. 3) and with year ($\chi^2 = 309.50$, df = 6, $P < 0.0001$); the most abundant species in 2009 and 2011 was *T. heterocerus*, in 2010 *P. morionellus*. *Diospilus capito* was present each year, but formed less than 25% of all parasitoids

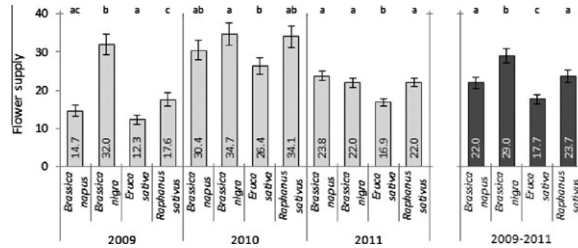

Fig. 1. Least square means (± standard error) of flower supply (mean number of flowers per plant). Different letters indicate statistically significant ($P < 0.05$) differences between cultures at the same year or over the whole study period 2009–2011 (according to Poisson model considering effect of culture and nonzero covariance between observations corresponding to the same replicate, and effect of year in the whole study period analysis).

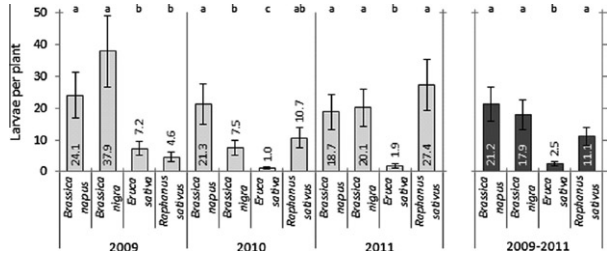

Fig. 2. Least square means (± standard error) of number of *M. aeneus* larvae per plant. Different letters indicate statistically significant ($P < 0.05$) differences between cultures at the same year or over whole study period 2009–2011 (according to Poisson model considering effect of culture and nonzero covariance between observations corresponding to the same replicate, and effect of year in the whole study period analysis).

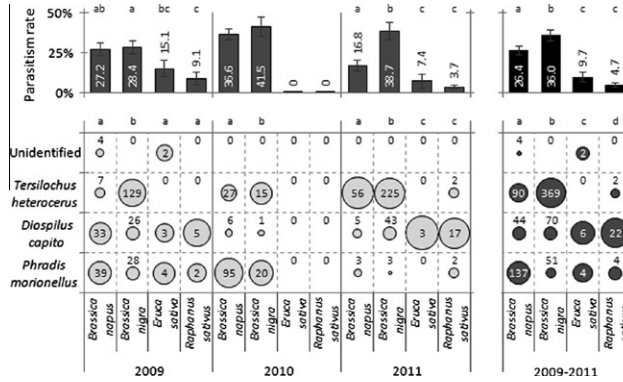

Fig. 3. Least square means (± standard error) of parasitism rate of *M. aeneus* larvae and species composition (circle size corresponding to the culture and year) and total numbers of *M. aeneus* larval endoparasitoids (numbers inside or above circles) found from 2nd instar larvae on different host plant flowers in 2009–2011. Different letters indicate statistically significant ($P < 0.05$) differences between cultures at the same year or over the whole study period 2009–2011 (according to logistic model considering effects of culture and year (only in the whole study period analysis) and nonzero covariance between observations corresponding to the same replicate in the parasitism rate comparison and Fisher exact test in the endoparasitoids' species composition comparison).

each year. The unidentified "healthy-fat-one" was present only in 2009 (Fig. 3). During the study all four parasitoid species were present on *B. napus*, *T. heterocerus*, the most abundant parasitoid species, was absent on *E. sativa* and the "healthy-fat-one" on *B. nigra* and *R. sativus* (Fig. 3). The largest number of parasitoids was recorded from *B. nigra*, followed by *B. napus*, with a few only from *E. sativa* and *R. sativus*.

Over the years, *P. morionellus* has exploited its potential heavily on *B. napus*, where it formed 50% of all parasitoids on this plant species, *D. capito* on *E. sativa* (50%) and *R. sativus* (79%) but *T. heterocerus* on *B. nigra* (75%).

4. Discussion

Visual and olfactory cues are both important in host plant location by phytophagous insects, including the pollen beetle (Blight and Smart, 1999; Cook et al., 2007a,b, 2013; Döring et al., 2012; Giamoustaris and Mithen, 1996). In this study we found, that, for oviposition, the beetle preferred *B. napus*, *B. nigra* and *R. sativus* over *E. sativa*. This concurs with results from laboratory trials that also showed the beetle preferred *B. nigra* and *B. napus* over *E. sativa* (Ekbom, 1998; Ekbom and Borg, 1996), confirming differences in attractiveness within the same plant family. But, as no differences between *B. napus* and *B. nigra* were detected, our results contrast with the laboratory studies of Borg (1996), Ekbom and Borg (1996) and Ulber and Thieme (2007), all of whom reported significantly fewer larvae on *B. nigra* than on *B. napus*. On the other hand, the only study conducted in field conditions has shown *B. nigra* to be considerably more attractive than *B. napus* to overwintered pollen beetle adults for feeding as well as for oviposition (Veromann et al., 2012). Also the attractiveness of *R. sativus*, which was similar to that of *Brassica* species, contrasts with the laboratory studies of Ekbom (1998), who found species from the genus *Brassica* to be more attractive to the beetle than crucifers from other genera.

Although pollen beetles are known to respond to yellow color during host location (Blight and Smart, 1999; Cook et al., 2006,

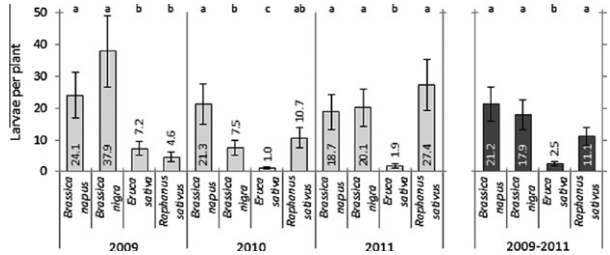

Fig. 2. Least square means (± standard error) of number of *M. aeneus* larvae per plant. Different letters indicate statistically significant ($P < 0.05$) differences between cultures at the same year or over whole study period 2009–2011 (according to Poisson model considering effect of culture and nonzero covariance between observations corresponding to the same replicate, and effect of year in the whole study period analysis).

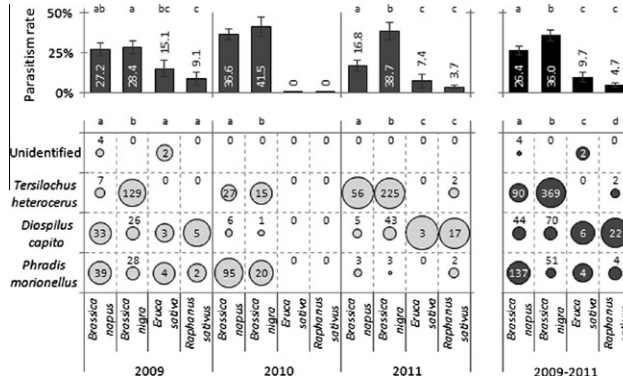

Fig. 3. Least square means (± standard error) of parasitism rate of *M. aeneus* larvae and species composition (circle size corresponding to the culture and year) and total numbers of *M. aeneus* larval endoparasitoids (numbers inside or above circles) found from 2nd instar larvae on different host plant flowers in 2009–2011. Different letters indicate statistically significant ($P < 0.05$) differences between cultures at the same year or over the whole study period 2009–2011 (according to logistic model considering effects of culture and year (only in the whole study period analysis) and nonzero covariance between observations corresponding to the same replicate in the parasitism rate comparison and Fisher exact test in the endoparasitoids' species composition comparison).

each year. The unidentified "healthy-fat-one" was present only in 2009 (Fig. 3). During the study all four parasitoid species were present on *B. napus*, *T. heterocerus*, the most abundant parasitoid species, was absent on *E. sativa* and the "healthy-fat-one" on *B. nigra* and *R. sativus* (Fig. 3). The largest number of parasitoids was recorded from *B. nigra*, followed by *B. napus*, with a few only from *E. sativa* and *R. sativus*.

Over the years, *P. morionellus* has exploited its potential heavily on *B. napus*, where it formed 50% of all parasitoids on this plant species, *D. capito* on *E. sativa* (50%) and *R. sativus* (79%) but *T. heterocerus* on *B. nigra* (75%).

4. Discussion

Visual and olfactory cues are both important in host plant location by phytophagous insects, including the pollen beetle (Blight and Smart, 1999; Cook et al., 2007a,b, 2013; Döring et al., 2012; Giamoustaris and Mithen, 1996). In this study we found, that, for oviposition, the beetle preferred *B. napus*, *B. nigra* and *R. sativus* over *E. sativa*. This concurs with results from laboratory trials that also showed the beetle preferred *B. nigra* and *B. napus* over *E. sativa* (Ekbom, 1998; Ekbom and Borg, 1996), confirming differences in attractiveness within the same plant family. But, as no differences between *B. napus* and *B. nigra* were detected, our results contrast with the laboratory studies of Borg (1996), Ekbom and Borg (1996) and Ulber and Thieme (2007), all of whom reported significantly fewer larvae on *B. nigra* than on *B. napus*. On the other hand, the only study conducted in field conditions has shown *B. nigra* to be considerably more attractive than *B. napus* to overwintered pollen beetle adults for feeding as well as for oviposition (Veromann et al., 2012). Also the attractiveness of *R. sativus*, which was similar to that of *Brassica* species, contrasts with the laboratory studies of Ekbom (1998), who found species from the genus *Brassica* to be more attractive to the beetle than crucifers from other genera.

Although pollen beetles are known to respond to yellow color during host location (Blight and Smart, 1999; Cook et al., 2006,

2013; Döring et al., 2012; Giamoustaris and Mithen, 1996), in the present study they were not influenced by flower supply i.e. more yellow plots. As olfactory cues are also key factors in their host location (Charpentier, 1985; Cook et al., 2002, 2006, 2007b; Evans and Allen-Williams, 1994; Ruther and Thiemann, 1997; Veromann et al., 2013) and insects favor using less energy to gain information (Fawcett and Johnstone, 2003), we can hypothetically assume that the beetle distinguishes between plant species firstly by their chemical composition, and uses visual cues secondarily to locate the most suitable host. For flying insects, olfactory cues are paramount as these can be detected in flight whilst avoiding excessive energy cost and predators (Pyke et al., 1977).

The parasitism rates of pollen beetle larvae were investigated on *B. nigra*, *E. sativa* and *R. sativus* for the first time. Compared to previous studies from several European countries where a parasitism rate exceeding 50% has been reported on *B. napus* (Austria: Kromp and Kraus, 2006; Finland: Hokkanen, 2006; Germany: Nitzsche and Ulber, 1998; Sweden: Nilsson, 1989; Switzerland: Büchi, 2002; UK: Williams, 2006), rates in the current study were considerably lower. On *B. napus*, it varied from 16.5 up to 33.6% over the years, which is smaller than was reported before in Estonia (48%, Veromann et al., 2013), but higher than found in conventional fields in Estonia (0–16%) (Veromann et al., 2006a,b). The variation of parasitism rates between other plant species was even higher: from 0% up to 38%. Spatial variation of parasitism rate depends on several different factors such as the availability of nectar as food source in concurrence with certain species, flower morphology, parasitoids' mandibles, ability to locate food sources (Gilbert and Jervis, 1998; Idris and Grafius, 1995; Jervis et al., 1993; Lee and Heimpel, 2003; Wäckers 2005) as well as on the enhanced fecundity of females which also depends on plant species (Idris and Grafius, 1995). Therefore, parasitism rate is highly conditioned by the local factors. In the current study parasitoids were supported most on *B. nigra*, whereas on *E. sativa* and *R. sativus* the parasitism rate was up to 10-fold lower than on *Brassica* species. This is also confirmed by Kaasik et al. (2013) who found higher parasitism rates of pollen beetle on *Brassica* species compared to another alternative host plant, *S. alba*. Therefore, based on our results, we conclude that parasitoids are attracted more to *Brassica* species than to other crucifer genera even when the latter support the development of pollen beetle larvae.

The species composition of the endoparasitoids of pollen beetle larvae on different host plant species were investigated for the first time. During the study, three key species of endoparasitoids were found: *T. heterocerus*, *P. morionellus* and *D. capito*; these are the most common and widely distributed species throughout Europe (Nilsson, 2003; Ulber et al., 2010). The host seeking behavior is similar in all three parasitoid species: they seek for hosts from buds and open flowers (Williams and Cook, 2010). The host finding success of female *T. heterocerus* on *B. nigra* were predominantly more effective than that of female *P. morionellus* and *D. capito*. These differences might be linked to their host searching behavior, as *T. heterocerus* explores flowers despite host abundance (Ferguson et al., 2003; Jönsson et al., 2004) but *P. morionellus* is attracted to the plants infested by pollen beetle larvae (Jönsson and Anderson, 2008). Larvae parasitized by *T. heterocerus* constituted over 75% of all infested larvae on *B. nigra*, thus, we can speculate that olfactory cues associated with the host and used by *P. morionellus* were less effective than the more visual search by *T. heterocerus*. On *B. napus*, *P. morionellus* outnumbered other parasitoid species: this concurs with Husberg and Hokkanen (2001) and Veromann et al. (2006d) who found *P. morionellus* to be the dominant species attacking the pollen beetle in Finland and Estonia, respectively. In general, the most abundant parasitoid species in our study was *T. heterocerus*, because it was extremely abundant on *B. nigra*. This was interesting, as in northern Europe, *T. heterocerus* is outnumbered by *P. morionellus* and *D. capito* on *B. napus* (Jönsson et al., 2004; Kaasik et al., 2013; Ulber et al., 2010; Veromann et al., 2006a–d) which were also found from all plant species in the current study. This indicates that *P. morionellus* and *D. capito* can efficiently locate alternative host plants. On non-brassica species, *E. sativa* and *R. sativus*, *D. capito* was the most efficient parasitoid species.

Thus, it is possible that *T. heterocerus* could more effectively locate suitable hosts on a host plant other than oilseed rape and therefore exhausted almost all (80%) of its oviposition potential on this alternative host plant. Less than 20% of all *T. heterocerus* specimens were found on *B. napus* indicating its lower attractiveness either due to unfavorable host abundance or better recognition of *B. nigra* than *B. napus*. Also, it may be possible that they were more attracted to volatiles emitted by *B. nigra*. In that case, it might be possible to use *B. nigra* to attract this parasitoid species and therefore to promote biocontrol in certain areas from where it could spread to oilseed rape fields. As the current study showed the parasitoids of the pollen beetle to be density independent, they might spread to other plant species in case of limited host larvae availability on *B. nigra*.

5. Conclusion

Our study shows that *B. nigra* has potential to reinforce the natural control of the pollen beetle. Not only was it attractive to this pest as an oviposition site, but it provided suitable hosts for parasitoid species not prevalent on oilseed rape. In addition to trapping pests, *B. nigra* could also act as a parasitoid bank, providing habitat for beneficial insects.

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II

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Abstract It is often suggested that weeds from the same family as the crop plant may increase insect pest damages by providing shelter and additional oviposition opportunities. We compared the relative attractiveness of *Brassica rapa* L., *B. juncea* L., *Sinapis alba* L. and *B. napus* L. (Capparales: Brassicaceae) to the pollen beetle and its hymenopteran parasitoids in field conditions. Our results revealed that none of the investigated plants increased the pest abundance on *B. napus* plants. On the contrary, *B. juncea* and *S. alba* lured beetles away from *B. napus* during its damage-susceptible stage. The parasitism rate of pollen beetle larvae was the highest on *B. juncea* plants, indicating that cruciferous weeds could improve the natural control of the pollen beetle by providing additional hosts for parasitoids. Therefore, close relatives of oilseed rape might be used to trap pollen beetle adults, but also to support populations of natural enemies that could decrease the number of beetles.

Keywords *Brassica napus* · *Meligethes aeneus* · Parasitoids · Attractiveness of host plants · Cruciferous weeds · Trap cropping

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Introduction

To achieve both social and economic satisfaction, agricultural sector needs to introduce sustainable production and management of arable crops. As conventional farming relies heavily on non-reproducible resources to produce energy, fertilizers and pesticides, sustainable alternatives are a necessity. Modern agriculture is also confronted with the problem of unwanted plants on the fields, especially the ones closely related to the main crop, which are harder to control and might serve as potential reservoirs of pests and diseases.

Oilseeds, which mainly consist of oilseed rape (*Brassica napus* L.: Capparales: Brassicaceae), are the most abundant cruciferous and the most important oil

crops cultivated in Europe (FAO 2013). The vast expansion of the growing area of *B. napus* over the last decades has created good preconditions for increasing pest populations of its pests (Hokkanen 2000). The most common insect pest of oilseed rape in Europe is the pollen beetle (*Meligethes aeneus* Fab., Coleoptera: Nitidulidae) which feeds on buds and flowers and therefore greatly reduces the seed yield (Alford et al. 2003; Cook and Denholm 2008; Veromann et al. 2006a, b, 2008; Williams 2010). Today, the management of pollen beetle is based on chemical control. This is a very unsustainable solution since in addition to reducing the economic competitiveness, pesticides induce the resistance in pests and kill their natural enemies (e.g. parasitoids) (Nilsson 1994; Pimentel et al. 1992; Theiling and Croft 1988), leading to even greater pest control problems (Veromann et al. 2008).

One of the ecologically more sustainable crop protection approaches involves exploiting insects' host plant seeking behaviour as a tool to direct the pests away from the crop of interest (Cook et al. 2006, 2007a; Veromann et al. 2012) or lure their natural enemies into the field (Landis et al. 2000). As related plants often share the same pests, other brassicaceous plants have a potential to be used in such environmental friendly crop protection systems of oilseed rape. However, there is a threat that these plants can provide suitable food source and shelter to the pollen beetle and therefore help to increase the populations of this pest. There is only limited information available about the attractiveness of different cruciferous plants to the pollen beetles. Some studies have reported that *Brassica rapa* is more attractive to *M. aeneus* than *B. napus* (Buechi 1990; Cook et al. 2006, 2007b). But as attractiveness of *B. rapa* relies at least partially on plant growth stages (Cook et al. 2007a, b) and the mentioned studies were conducted in Central Europe, the question remains whether *B. rapa* or other cruciferous plants could be also used as trap crops in Northern Europe where climatic conditions are different and vegetation period is shorter. Since plant growth is heavily influenced by environmental conditions, the efficiency of trap crops depends on the climate conditions where it has been developed and the development of optimal systems for different climatic regions is essential.

Since all cruciferous plants could be potential oviposition hosts for pollen beetles, they can also serve as sites for building up the populations of the larval parasitoids of these pests. In favourable conditions, the parasitism rate of *M. aeneus* has been reported to be as high as 97 % (Ulber et al. 2006, 2010) which indicates their capability to significantly control the pollen beetle population. A choice of host plants could also diversify the species composition of parasitoids (Kovács et al. 2013), which could be important for stable and balanced natural pest control. For example, Billqvist and Ekbom (2001a,b) showed that host plant species has a minor effect on the parasitism rate of *M. aeneus* by the parasitoid *Phradis morionellus* Holm., whereas the parasitism by *Diospilus capito* Nees did depend on plant species. Other than these results, the dependence of parasitoids' species composition on different host plant species has not been investigated and needs further attention to facilitate our understanding of parasitoids' behaviour.

The aim of this study was to assess the relative attractiveness of the four most common cruciferous plants in Europe, *B. rapa*, *B. juncea*, *Sinapis alba* and *B. napus* (Capparales: Brassicaceae), to the pollen beetle and to determine whether the host plant influences host finding success of parasitoids and/or their species composition. We demonstrate that plant species considered as weeds may lure pests away from oilseed rape during its most susceptible stages and have positive effect on beneficial insects, such as parasitoids, by providing alternative host plants and fostering the same but as well different species of parasitoids than *B. napus*.

Materials and methods

Field experiment setup

The field study was carried out in the experimental field of the Estonian University of Life Sciences in Tartu, Estonia (58°21′N, 26°39′E) from 2006 to 2008. The plants from four different cruciferous species were grown in a randomized complete block design of three replicates with the plot size of 1 × 5 m. Each plot had a 1 m wide buffer zone of bare soil around them to minimize inter-plot interactions. Plots were sown on 5th of May in 2006 and 2007, and on 15th of May in 2008, with the seed of *B. napus*, *B. rapa*, *B. juncea* and *S. alba*, with the density of 250 seeds per m^2. In 2006, seeds of *B. juncea* were purchased from Hansaplant LLC (Tartu, Estonia), and the seeds of *B.*

napus, *B. rapa* and *S. alba* were obtained from the seed collection of the Estonian University of Life Sciences. In the following years, seeds collected from the experimental plots in the autumn of the previous year were used. Over all three study years, crop management was uniform in all plots: no pesticides and fertilizers were used. Plant growth stage (BBCH) was determined every sampling day (see Table 1) using the decimal code system of Lancashire et al. (1991).

Quantification of pests and their parasitoids

To determine plant attractiveness to *M. aeneus* adults, beetles were counted from all used plant species at various growth stages. The assessments were started when *B. napus* plants were forming side shoots (BBCH 23), performed in average every 3–4 days and finished at the end of flowering (BBCH 67), totalling nine assessments each year. Due to variable weather conditions in different years, the attractiveness of different plant species to *M. aeneus* adults was determined by grouping data from all plant species over the years based on growth stages of *B. napus* at each collection date and marked as sampling 1, sampling 2 etc. (Fig. 1). Beetles were collected from the flowers of ten randomly chosen plants from each plot using the beating method (Williams et al. 2003). Insects were counted and identified in the laboratory, with an Olympus SZ-CTV stereo microscope (Olympus Optical CO, LTD, Japan) with a 12.5× objective.

The oviposition preferences of *M. aeneus* were determined at the full flowering stage (BBCH 65–67; Table 1) of each plant species when all larvae from the flowers of ten randomly chosen plants per plot were collected. The parasitism level of *M. aeneus* larvae was determined by larval dissection. In the current study, all second instar larvae were dissected and parasitoids' eggs and/or larvae were counted and identified to species level according to the key by Osborne (1960).

Data analysis

Statistical analyses were carried out using GLM and GENMOD procedure in SAS 8.02 (SAS Institute, Inc., Cary, NC, USA). The differences in mean numbers of *M. aeneus* larvae, *M. aeneus* adults per sampling time and plant species were analysed by using Wald

Table 1 The sampling dates and the corresponding growth stages (BBCH) (Lancashire et al. 1991) of tested plant species at different sampling times in 2006–2008

	Sampling times								
	Year	1	2	3	4	5	6	7	8	9
	2006	06/06	08/06	13/06	16/06	20/06	22/06	27/06	30/06	04/07
	2007	08/06	11/06	14/06	18/06	21/06	25/06	28/06	02/07	05/07
	2008	19/06	23/06	26/06	30/06	02/07	07/07	14/07	17/07	21/07
Growth stages at each sampling time										
Brassica napus	2006	21–23	23–31	35–55	35–55	55–59	55–59	64–66	66–67[a]	66–67
	2007	21–23	23–31	35–55	51–55	55–59	61–64	64–66	66–67	66–67[a]
	2008	21–23	23–31	35–55	51–55	55–59	61–64	64–66	66–67[a]	66–71
Brassica rapa	2006	32–51	55–57	57–59	67–69	67–69[a]	69–71	69–71	71–75	81–83
	2007	32–51	55–57	57–59	61–67	67–69	67–69[a]	69–71	71–75	71–75
	2008	32–51	55–57	57–59	61–67	67–69	67–69[a]	69–71	71–75	81–83
Brassica juncea	2006	31–33	61–63	61–63	61–63	65–67[a]	69–77	71–77	71–77	71–77
	2007	31–33	61–63	61–63	63–65	65–67[a]	69–77	71–77	81–83	81–83
	2008	31–33	61–63	61–63	63–65	65–67	65–67[a]	71–77	81–83	85–90
Sinapis alba	2006	31–33	61–63	61–64	61–64	65–67[a]	67–74	71–74	71–74	75–79
	2007	57–59	61–63	61–64	63–65	65–67	67–74[a]	71–74	75–79	85–90
	2008	31–33	61–63	61–64	63–65	65–67	67–74[a]	71–74	71–79	85–90

[a] Indicates sampling time of *Meligethes aeneus* larvae each year

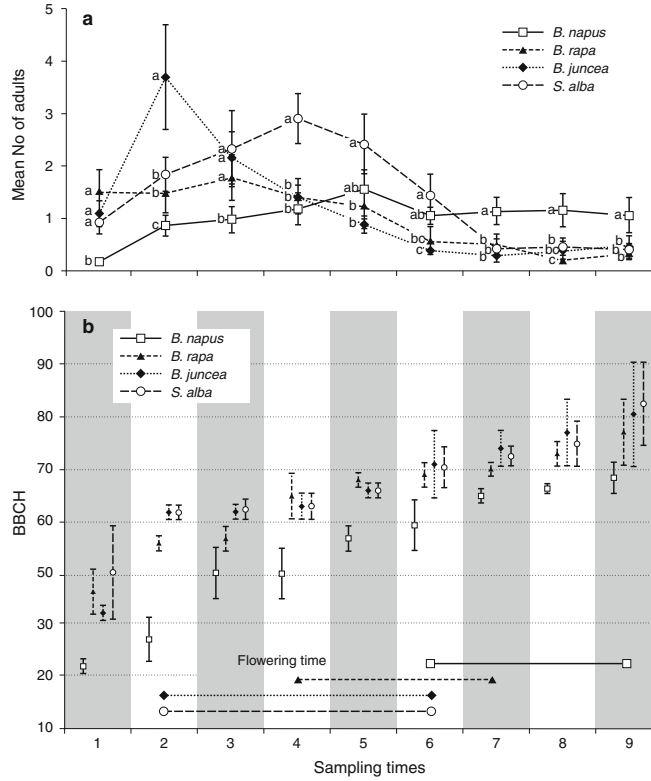

Fig. 1 Mean (±SE) number of *Meligethes aeneus* adults per plant at each survey (**a**) and the growth stages of plant species at each survey time in 2006–2008 (**b**). Different letters indicate statistically significant differences between plant species, $P < 0.05$; Wald statistics, Type III GENMOD Procedure, SAS. Phenological growth stages using BBCH-scale (Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie) according to Lancashire et al. (1991): 21 beginning of side shoot development, 21–27 side shoots 1–7 detectable, 27–29 end of side shoot development, 30–39 stem elongation, 50 buds enclosed, 51–52 green buds, 53–55 buds extending, 55–59 yellow buds, 60–65 first flower–50 % flowering (full flowering), 67 flowering declining, 69 end of flowering, 71–79 development of fruit, 80–89 ripening

statistic Type III empirical standard error analysis with the Poisson distribution and the log link function. For those analyses, the mean numbers were pooled over the three study years. Differences of the mean number of adult beetles between the tested plant species were found using the GENMOD procedure Differences of Least Squares Means test. Comparisons of the number of parasitized *M. aeneus* larvae were made using the same analysis, but with Binomial distribution and logit link function, in which the response variable was the number of dissected larvae. The scale parameter was estimated by Pearson χ^2 divided by the degrees of freedom to account for the model over-dispersion. To discover the false discovery rate (FDR) the multiple comparison adjustments (FDR procedure in SAS) were used. The Pearson correlation analysis was used to assess the relationship between larval abundance and parasitism rate.

Results

Abundance of *M. aeneus* adults

Meligethes aeneus adults were found from all studied plant species and in every study year. Although the mean number of beetles per plant was rather small, the plant species had significant influence on their abundance over the years ($\chi^2 = 62.57$, df = 3, $P < 0.0001$). The highest number of beetles was found on *S. alba* plants (in average 1.47 ± 0.16 per plant), which is significantly greater compared to the adults found on *B. juncea* ($\chi^2 = 29.10$, df = 1, $P < 0.0001$), *B. napus* ($\chi^2 = 24.60$, df = 1, $P < 0.0001$) and *B. rapa* ($\chi^2 = 50.78$, df = 1, $P < 0.0001$). Beetles' abundance was the lowest on *B. rapa* (1.02 ± 0.11 beetles per plant) followed by *B. juncea* (1.16 ± 0.18; $\chi^2 = 6.06$, df = 1, $P = 0.014$) and *S. alba* ($\chi^2 = 50.78$, df = 1, $P < 0.0001$). No differences were found between the beetles' abundance on *B. napus* (1.15 ± 0.09) compared to *B. juncea* ($\chi^2 = 0.58$, df = 1, $P = 0.45$) and *B. rapa* ($\chi^2 = 1.79$, df = 1, $P = 0.18$).

When comparing the beetles' abundance at different sampling times, significant differences were detected (see Fig. 1 for full statistical analyses). The least preferred plant species during the first three sampling times was *B. napus* ($P < 0.05$; Fig. 1). At the fourth sampling time when *B. napus* was in the vulnerable green/yellow bud stage, there were significantly more beetles on *S. alba* plants than on all other plant species. At this time, the abundance of beetles on *S. alba* was significantly higher than on *B. rapa* ($\chi^2 = 13.38$, df = 1, $P = 0.0003$), *B. juncea* ($\chi^2 = 13.83$, df = 1, $P = 0.0002$) and *B. napus* ($\chi^2 = 18.77$, df = 1, $P < 0.0001$); *B. napus*, *B. rapa* and *B. juncea* had similar numbers of *M. aeneus* per plant.

In samplings 5 and 6, the distribution of beetles between the plant species had changed. At these sampling times, the abundance of beetles on *S. alba* had decreased but its attractiveness still remained higher compared to *B. rapa* (sampling 5: $\chi^2 = 6.93$, df = 1, $P = 0.0085$; sampling 6: $\chi^2 = 9.26$, df = 1, $P = 0.0023$) and *B. juncea* (sampling 5: $\chi^2 = 12.96$, df = 1, $P = 0.0003$; sampling 6: $\chi^2 = 14.14$, df = 1, $P = 0.0002$; Fig. 1). Thereafter, since the beginning of the full flowering stage of *B. napus* (BBCH 65; Samplings 7–9), pollen beetles clearly preferred *B. napus* over all the other studied plant species ($P < 0.01$; Fig. 1).

Abundance of *M. aeneus* larvae

When comparing the oviposition preferences of *M. aeneus*, an influence of the hosts' species was found to be significant ($\chi^2 = 103.41$, df = 3, $P < 0.0001$; Fig. 2). *Brassica napus* was preferred over *B. rapa* ($\chi^2 = 32.30$, df = 1, $P < 0.0001$), *B. juncea* ($\chi^2 = 31.47$, df = 1, $P < 0.0001$) and *S. alba* ($\chi^2 = 72.74$, df = 1, $P < 0.0001$). The least preferred plant species for oviposition was *S. alba* where fewer larvae were found also compared to *B. juncea* ($\chi^2 = 31.58$, df = 1, $P < 0.0001$) and *B. rapa* ($\chi^2 = 30.99$, df = 1, $P < 0.0001$).

Parasitism rate of *M. aeneus* larvae

The parasitism rate of *M. aeneus* also depended on the plant species ($\chi^2 = 16.48$, df = 3, $P = 0.0009$) and was not correlated with the abundance of hosts ($r = 0.11$, $n = 229$, $P = 0.12$). Although the larvae from all plants were highly parasitized (41–65 %) the ones from *B. juncea* flowers had greater parasitism rates than those of *B. napus* when the data were pooled over the years ($\chi^2 = 13.49$, df = 1, $P = 0.0002$) as well as when the data were analysed separately for each year.

Species composition of *M. aeneus* larval endoparasitoids

The abundance and representation of parasitoid species differed between plant species. In total, three parasitoid species were found from *M. aeneus* larvae: *D. capito*, *P. morionellus* and *Tersilochus heterocerus* Thom. (Fig. 3). All three species were represented each year, with *P. morionellus* being the most dominant and *D. capito* the second most numerous. Over the course of the study, the relative occurrence of *T. heterocerus* was the most uniform over all plant species (Fig. 3). The most unequally distributed parasitoid species was *D. capito* which formed 44.6 % of all specimens on *B. napus* but represented only 7.1 % of all parasitoids on *S. alba* due to the absence in 2007 and 2008.

Discussion

Feeding and oviposition preferences of *M. aeneus*

The only plant species found to be more attractive to *M. aeneus* adults than *B. napus* was *S. alba*. *Brassica*

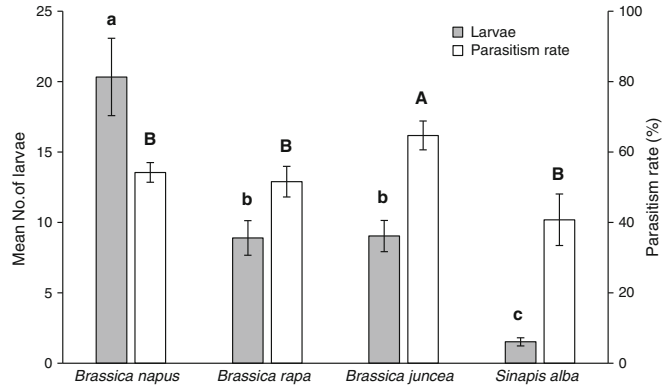

Fig. 2 Mean (±SE) number of *Meligethes aeneus* larvae per plant (grey bars), its parasitism rate (±SE) (white bars) on different host plant species in 2006–2008 study period (different letters indicate statistically significant differences between plant species (lowercase letters indicate differences between the mean number of *Meligethes aeneus* larvae per plant, capital letters indicate differences between parasitism rate). Larvae per plant: $P < 0.0001$, parasitism rate: $P < 0.05$; Wald statistics, Type III GENMOD Procedure, SAS

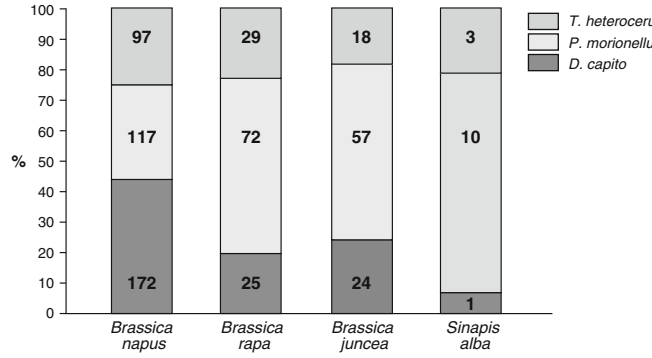

Fig. 3 Species composition and total numbers of *Meligethes aeneus* larval endoparasitoids (*Tersilochus heterocerus*, *Phradis morionellus* and *Diospilus capito*) on different host plant species in 2006–2008 study period

rapa was only found more attractive than *B. napus* during the beginning of its bud stage when *B. napus* was still at the stem-elongation stage with buds present. These results differ from previous studies that have shown a greater attractiveness of *B. rapa* compared to *B. napus* in laboratory as well as in field conditions (Buechi 1990; Cook et al. 2006, 2007b; Rusch et al. 2010). These preferences depend at least partially on plant growth stages (Cook et al. 2007b). Dissimilarities to previous studies might be caused by different climate conditions, as our study was carried out in northern European climate with shorter vegetation period compared to southern European or maritime climate where other studies were conducted.

When sampling times were included in the analyses, the results also showed changing attractiveness over time. Although the developmental speed of *B. rapa*, *B. juncea* and *S. alba* plants was rather synchronous it did not lead to equal attractiveness to *M. aeneus* beetles which indicates that plant species in the same genus may have different attractiveness to *M. aeneus* adults during similar growth stages at the same time.

As current results show, from the start of green bud stage, which is the most damage-susceptible growth stage (BBCH 50–59) of *B. napus* (Nilsson 1994; Williams and Free 1979), *B. rapa* did not attract *M. aeneus* despite its more advanced growth stage. Therefore, we may conclude that *B. rapa*, although showing suitable features in southern Europe and in the UK (Buechi 1990; Cook et al. 2006, 2007b), may not be a suitable trap crop for the pollen beetle in climate conditions similar to Estonia. Veromann et al. (2012) found that *B. nigra* can be used as a potential for trap crop for spring variety of *B. napus* in the Baltics. The greater attractiveness of *S. alba* compared to *B. napus* indicates for the first time that *S. alba* could be used as a trap crop. As it may serve as an earlier food source for *M. aeneus* adults, its availability on the field could decrease the yield loss caused by adult beetles feeding on buds of *B. napus*.

In the current study, the most preferred species for oviposition was *B. napus* and the abundance of larvae was the lowest on *S. alba*. This supports previous studies that indicate differences in the oviposition attractiveness in the genus *Brassica* (Veromann et al. 2012) and that *Brassica* species are more attractive than *S. alba* (Borg and Ekbom 1996; Ekbom 1998). It is likely that the attractiveness of the plants for egg laying is determined by other factors than the availability of suitable buds because there were plenty of buds available on *S. alba* plants.

Based on these results we can conclude that only some close relatives of *B. napus* are more attractive and offer oviposition sites for *M. aeneus*. These plants provide pollen for feeding and therefore can prevent damage on *B. napus*, but majority of these will not support oviposition and hence will not increase the number of the future generations. However, as the plot sizes in the current study were too small to draw fundamental conclusions about the behaviour of *M. aeneus* in large fields, additional large scale field experiments are necessary to confirm this assumption. Although the presence of insect pests may be encouraged by the presence of wild host plants in the vicinity of cultural fields (van Emden 1965; Norris and Kogan 2005), in the present study no evidence of related plant species to increase beetle abundance was found.

Abundance and species composition of parasitoids

The parasitism rates of *M. aeneus* in the current study were up to 63 % which is remarkably higher than reported before in Estonia (up to 48 %; Veromann et al. 2013). As parasitism rates of 30–40 % can effectively decrease the abundance of *M. aeneus* (Hokkanen 2008) we can hypothesize that parasitoids should be able to control the pests' abundance on the spring variety of *B. napus*.

While the parasitism rates between *B. napus* and *S. alba* were similar, the host availability was significantly greater on *B. napus*. As no correlation between *M. aeneus* abundance and its parasitism rate was found, we are inclined to think that the host seeking success of *M. aeneus* parasitoids as a complex does not depend on host density. Additionally, when comparing the parasitoid attractiveness between *B. juncea* and all other tested plants, parasitoids' host seeking behaviour was more effective on *B. juncea.* Therefore, the presence of *B. juncea* nearby the oilseed rape fields could enhance the effectiveness of biological control by supporting parasitoids. However, additional large field experiments are needed to test whether the potential of *B. juncea* is also revealed when planted in the proximity of *B. napus* fields.

Meligethes aeneus larvae were parasitized by *D. capito, P. morionellus and T. heterocerus*, which is congruent with previous studies in Europe (Hokkanen 1989; Nilsson 2003; Nilsson and Andreasson 1987; Osborne 1960; Veromann et al. 2006a, b, c). Surprisingly, despite advantages such as earlier arrival to the field (Nilsson 2003) and superiority over *T. heterocerus* in case of multiparasitism (Nitzsche and Ulber 1998), another common parasitoid of *M. aeneus*, *P. interstitialis*, which has been recorded throughout Europe (Nilsson 2003), was not found in the current study. According to the study of Ferguson et al. (2003) in the UK, the lack of *P. interstitialis*, despite its advantages, might be caused by winter mortality as the survival rate to adulthood for this species is only 1.5 %. In addition, its absence in current study might be related to its earlier arrival to the field (Nilsson 2003; Williams 2006) or hosts' size preferences. As *P. interstitialis* oviposits into the eggs and first instar larvae (Nilsson 2003) it is possible that during the collection time of *M. aeneus* larvae, the larvae parasitized by *P. intersitialis* had already dropped from plants to the soil to pupate.

Over 50 % of available larvae can be parasitized by univoltine species: *P. interstitialis*, *P. morionellus* and *T. heterocerus* (Nilsson 2003). In the current study more than 75 % were used by only two species: *P.*

morionellus and *T. heterocerus*. These species gained superiority on *B. rapa*, *B. juncea* and *S. alba* each year but on *B. napus* the prevalent parasitoid species was *D. capito*. Although Billqvist and Ekbom (2001a) found greater risk of parasitization by *D. capito* on *S. alba* compared to *B. napus,* our results, also supported by Veromann et al. (2006a), show that multivoltine *D. capito* is capable of controlling a significant amount of *M. aeneus* on *B. napus* and its importance is probably underestimated.

Diospilus capito does not discriminate hosts by their size (Osborne 1960) nor previous parasitization (Nilsson and Andreasson 1987), it is privileged in the case of multiparasitism (Jourdheuil 1960) and has several generations per year. Therefore, we can assume that its lower representation on plant species other than *B. napus* is based on its poorer host location success on alternative host plants rather than on the abundance of host larvae. In addition, the interspecific competition between parasitoid species for host resources can also play a key role that influences the species composition. For instance, Cusumano et al. (2011) have shown that the coexistence of parasitoid species can be directed by both interspecific extrinsic and intrinsic competitive interactions, i.e. one egg parasitoid species was more efficient in host location while the other was successful at larval competition. Additionally, as the parasitoids species composition recorded on *B. rapa* and *B. juncea* was different from that on *B. napus*, we can hypothesize that plant species even within the same family might substantially alter parasitoids species composition.

All investigated plant species supported all detected parasitoid species. From this we can assume that alternative plants may be able to support the survival of parasitoids in case of reaching the fields at a time when suitably sized hosts are not yet available on *B. napus*. They can also provide food for parasitoids, as Rusch et al. (2013) have shown that *T. heterocerus* can benefit from the presence of flowering plants in the oilseed rape field or in the surrounding landscape through nectar feeding. The adult *P. morionellus* only survive for a week in summer temperatures (Nilsson 2003), which limits their parasitization possibilities. Plant characters can also alter the host selection by both pests and their parasitoids directly or indirectly (Vinson 1976; Wang et al. 1997) and thereby also the searching efficiency of their natural enemies. As *T. heterocerus* searches hosts mainly from open flowers (Jönsson et al. 2005; Winfield 1963) the reason for its greater proportion on *B. napus* compared to other plant species might be caused by the more abundant yellow colour due to a more concentrated and intense flowering. Volatiles are essential cues for hosts' habitat location and differ between plant species. For many herbivorous insects specialised on glucosinolate-containing plants, glucosinolates serve as cues for feeding and oviposition (Mewis et al. 2002; Miles et al. 2005) as their breakdown products provide both long distance and contact information (Radojčić Redovniković et al. 2008). The type of glucosinolate and its breakdown products vary between plant species and cultivars (Clossais-Besnard and Larher 1991) and on winter varieties of *B. napus* the volatile bouquet may even differ with fertilisation level (Veromann et al. 2013). Therefore we can assume that provided volatile information may follow the same trend.

In conclusion, our results show that *S. alba* was more attractive during the most damage susceptible stage of oilseed rape but *B. juncea*, *B. rapa* and *S. alba* did not lure more pests to the field than *B. napus* itself. In contrary, they can even prevent damage by providing pollen for feeding but will not increase the number of offspring of pollen beetles as the majority of these plant species did not support oviposition. They can be exploited as entomological banks to enhance the presence of beneficial insects as the hymenopteran parasitoids associated with *M. aeneus* revealed their great host seeking efficiency even if very few hosts were available. *Brassica juncea* lured more parasitoids demonstrating its suitability for the major group of natural enemies of *M. aeneus*. Therefore, if parasitoid species will spread from *B. juncea* to *B. napus*, it might have a great potential for biological control. To understand the mechanisms behind the host selection of parasitoids, further large scale field studies as well as laboratory experiments are needed.

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III

Veromann, E., Metspalu, L., Williams, I.H., Hiiesaar, K., Mand, M., **Kaasik**, **R**., Kovacs, G., Jogar, K., Svilponis, E., Kivimagi, I., Ploomi, A., Luik, A. 2012. Relative attractiveness of *Brassica napus*, *Brassica nigra*, *Eruca sativa* and *Raphanus sativus* for pollen beetle (*Meligethes aeneus*) and their potential for use in trap cropping.

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ORIGINAL PAPER

Relative attractiveness of *Brassica napus*, *Brassica nigra*, *Eruca sativa* and *Raphanus sativus* for pollen beetle (*Meligethes aeneus*) and their potential for use in trap cropping

E. Veromann · L. Metspalu · I. H. Williams · K. Hiiesaar · M. Mand · R. Kaasik · G. Kovács · K. Jogar · E. Svilponis · I. Kivimagi · A. Ploomi · A. Luik

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Abstract Oilseed rape (*Brassica napus*) is a valuable crop, attacked by several insect pests, of which the pollen beetle (*Meligethes aeneus*) is one of the most widespread and important in Europe. The relative attractiveness for the pollen beetle of *Brassica nigra*, *Eruca sativa* and *Raphanus sativus* was compared with that of spring oilseed rape, to assess the potential of these plant species as trap crops for the pest. At early growth stages, *B. nigra* and *R. sativus* were more attractive to over-wintered adult pollen beetles than *B. napus*. At the bud and flowering stages, *B. nigra* was the most attractive while *E. sativa* was the least attractive. At flowering, *B. nigra* was more attractive for oviposition than the other species. Thus, of the species tested, *B. nigra* has the most potential as a prospective trap crop to protect spring oilseed rape before flowering when it is at its most vulnerable developmental phase, due to its faster development and its acceptability both for feeding and oviposition to overwintered pollen beetle adults. *Raphanus sativus* was accepted both for feeding and oviposition, but pollen beetle larvae failed to develop in its flowers; it therefore has the potential for use as a dead-end crop. At the end of the growing season, both *E. sativa* and *R. sativus*, as late-flowering species, attracted the new generation of pollen beetles and thereby have potential to extend the effectiveness of a trap-cropping system at this time.

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Keywords Host plant attractiveness · Pollen beetle · Adult and larval abundance

Introduction

Oilseed rape (*Brassica napus* L.) is increasing in importance as a crop throughout the world; its oil is used for cooking and more recently for bioenergy, while the meal is fed to livestock. It has become the sixth most important crop in Europe with nearly 8,787,770 ha under cultivation and the second most important oilseed crop after sunflower seed, mostly grown in France, Germany, Ukraine, Poland and in the UK (FAOSTAT 2010).

In Estonia, the sown area of the crop has increased 60-fold over the past 15 years; spring oilseed rape (*B. napus* L. var. *oleifera* subvar. *annua*) prevails over winter rape (*B. napus* L. var. *oleifera* subvar. *biennis*) because autumn sowing dates are unfavourable and winter rape is susceptible to frost damage. With the expansion of the area of oilseed rape grown, there has been an increase in the damage caused by insect pests, especially by the pollen beetle (*Meligethes aeneus* Fabricius) (Veromann et al. 2006a, b, 2008), one of the major pests of the crop throughout Europe (Ekbom 2010; Williams 2010).

Pollen beetles are univoltine. They overwinter as adults in the soil, leaf litter and herbaceous vegetation of field and forest borders (Büchi 2002). On emergence from hibernation in the spring, they are polyphagous, feeding on the pollen of spring-flowering plant species from different families until the females attain sexual maturity. For mating and oviposition, the beetles move to cruciferous plant species, including oilseed rape (Fritzsche 1957; Williams and Free 1978; Ekbom and Borg 1996). Females lay their eggs in the flower buds, preferring buds 2–3 mm long

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(Nilsson 1988; Borg 1996). Feeding by adults causes bud abscission (podless stalks) with yield reductions up to 70 % reported in spring oilseed rape (Nilsson 1987). New-generation beetles emerge in late summer and feed polyphagously before seeking overwintering sites (Williams and Free 1978).

In recent decades, control of the pollen beetle has relied largely on the pyrethroid class of insecticides, most often applied routinely and prophylactically, without regard to pest incidence (Williams 2004; Thieme et al. 2010). This has led to their over-use, reducing the economic competitiveness of the crop and threatening biological diversity. The pesticides also kill beneficial insects which help control the pests (Williams and Murchie 1995; Veromann et al. 2011). In addition, Hokkanen (2000) found that insecticide treatment at or above the threshold level for control did not significantly reduce the size of the new generation of pollen beetle that emerged and Veromann et al. (2008) even increased it. Further, the most serious current concern is the recent and widespread development of resistance to pyrethroids of the pollen beetle (Thieme et al. 2010).

The need to reduce dependence on chemical inputs for insect pest management is encouraging investigation into alternative options for control through the development of more ecologically sustainable and economically viable integrated crop management (Williams 2004, 2010; Cook and Denholm 2008; Nilsson 2010; Rusch et al. 2010). A potentially useful component in this system is the exploitation of host plant preferences (Cook et al. 2006b, a). The aim of this study was to compare the relative attractiveness of three cruciferous oilseed plants, *Brassica nigra* L. (black mustard), *Eruca sativa* L. (rucola) and *Raphanus sativus* L. *ssp. oleifera* (oilseed radish) with that of *Brassica napus* L. (spring oilseed rape), to the pollen beetle for feeding and oviposition and to determine their potential for use within a trap crop system to reduce the number of pollen beetles on spring oilseed rape. *Brassica nigra* is an annual species with yellow flowers, cultivated for its seeds, commonly used as a spice; it is not grown commercially in Estonia, but is locally naturalized. *Eruca sativa* is an erect annual species, with dark green, lobed leaves and pale yellow flowers with some brown veins; it is cultivated as an edible salad vegetable for the distinct spicy flavour of its young leaves and as an industrial non-food crop for the high erucic acid content of its oil (Lazzeri et al. 2004). *Raphanus sativus* is gaining importance in Europe as forage and green manure and is being tested in Estonia for use in *Brassica*–cereal rotations; it is an excellent scavenger of nitrate from deeper soil layers (Ngouajio et al. 2004). It has pale violet flowers.

Materials and methods

Study area and experimental design

Studies were carried out in an experimental field of the Estonian University of Life Sciences in 2009 and 2010. The plants were grown in a randomized complete block design with three replicates of each plant species: *B. napus* (cv. '*Mascot*'), *B. nigra, E. sativa* (cv. '*Poker*') and *R. sativus* (cv. '*Bille*'). In 2009, seeds of *B. nigra* and *E. sativa* were purchased from the seed company Hansaplant LLC, and seeds of *B. napus* and *R. sativus* were obtained from the seed collection of the Estonian University of Life Sciences. In 2010, seeds collected in the autumn from plants in the 2009 experiment were used. In both years, each plot was 1 m × 5 m with a 1-m wide buffer zone of bare soil around each plot to minimize inter-plot interactions; the whole experimental field was surrounded by spring barley. Plots were sown concurrently on 7 May 2009 and 12 May 2010, at 250 seeds per m^2. Crop management was uniform in all plots. Plant growth stage (GS) was assessed weekly in 2009 and every 3–4 days in 2010 using the decimal code system of Lancashire et al. (1991). To compare total flower production capacity of each plant species, the numbers of pods (successfully developed flowers) and podless stalks (flowers which had not completed their development into pods because of lack of pollination or damage including that caused by *M. aeneus*) were counted on ten randomly chosen plants from each plot at the pods' maturation stage (GS 80–83; from *B. nigra* on 11 August, *B. napus* and *R. sativus* on 18 August and *E. sativa* on 15 September 2009).

Insect sampling

Adult pollen beetles were sampled once a week in 2009 and every 3–4 days in 2010 in mid-morning from 26 May to 8 September 2009 and from 1 June to 31 August 2010. At the early growth stage (GS 12–39), the beetles from each of ten randomly chosen plants from each plot were collected into separate vials using an aspirator or pooter (a simple mouth-sucking apparatus consisting of a glass jar and two plastic tubes). At inflorescence emergence (GS 50–59 "green" and "yellow bud") and flowering stages (GS 60–69), the beating method was used (Williams et al. 2003). The main raceme of each of ten randomly chosen plants was tapped three times over a plastic tray (28 cm × 22 cm × 9 cm) to dislodge the beetles from the buds and flowers; the beetles from each plant were then collected from the tray into a separate vial with an aspirator, labelled and, in the laboratory, the species identified (using the binocular Olympus SZ-CTV and the key of

Kryzhanovsky (1965)) and counted. Most beetles were identified as *M. aeneus* and this is the species referred to as pollen beetle in this paper. After mid-June, a few specimens of *M. viridescens* were found occasionally, but since their abundance never exceeded 2–3 % of the sampled beetles, they were excluded from the data analyses. Insect samples captured between GS 12–65 (two leaves unfolded and flowering stages) were recorded as the overwintered generation, while those from 7 weeks after the first catch of the overwintered generation until the end of sampling were recorded as the new generation. New summer generation pollen beetle adults were sampled from *R. sativus* and *E. sativa* only, as *B. napus* and *B. nigra* completed flowering before their emergence. All beetles on a single flowering branch (at the same GS) of both plant species from each of ten plants randomly selected per plot were counted until the beetles moved away to their overwintering sites.

Larval abundance was determined by two methods: (1) by counting the larvae that dropped from flowers when adult beetles were assessed using the beating method, and (2) by dissecting buds and flowers under a microscope and counting the larvae (both instars) found in the flowers and buds of ten randomly chosen plants per plot at full flowering stage (GS 64–67) of each species (from *B. napus* and *B. nigra* on 7 July and from *R. sativus* and *E. sativa* on 14 July in 2009 only). To estimate egg abundance, 25 green buds (GS 51–52; from *B. napus*, *B. nigra* and *R. sativus* on 25 June and from *E. sativa* on 30 June in 2009 only) were collected from randomly chosen plants on each plot. In the laboratory, they were dissected under a microscope and eggs, infested and uninfested buds were counted.

Weather conditions

In 2009, spring/summer weather conditions were similar to the long-term average: May 11.3 °C (long-term average 10.1 °C), June 13.6 °C (14.5 °C), July 16.7 °C (16.7 °C) and August 14.9 °C (15.8 °C; EMHI, 2011). In May 2010, there were 2 weeks of warmer than average weather, followed by a period with average temperatures until the end of June (average temperatures in May 12.1 °C, June 14.3 °C). In July 2010, the average temperature was higher (21.7 °C, with temperatures over 30°C for more than 10 days) than the long-term average (16–17 °C). Precipitation was similar to the long-term average in both years (http://pk.emu.ee/struktuur/rohu-katsejaam/katsejaama-ilmastikuandmed/) (Meteos Compact Rõhu 2009, 2010).

Statistical analyses

Data were analysed using STATISTICA 9.1 (StatSoft, Inc.). The distribution of data was tested for normality by the Shapiro–Wilk W statistics (Zar 1996) and log transformed if necessary. ANOVA and LSD post hoc tests ($P \leq 0.05$) were applied to determine the differences between the numbers of pollen beetle adults (both overwintered and new generation), their eggs and larvae on the tested plant species. Separate analyses were conducted for each date and for different plant growth stages. To estimate the general impact of plant species on the abundance of pollen beetles during the most susceptible growth stages (BBCH 51–61) of *B. napus*, the average number of pollen beetles on each plant species (at GS 51–61) was calculated. To estimate the potential capacity to produce flowers during the whole sampling period of tested plant species, the sum of the number of pods and podless stalks per plant was calculated. To test for differences among all plant species, univariant analysis of variance (ANOVA) and post hoc LSD test were used.

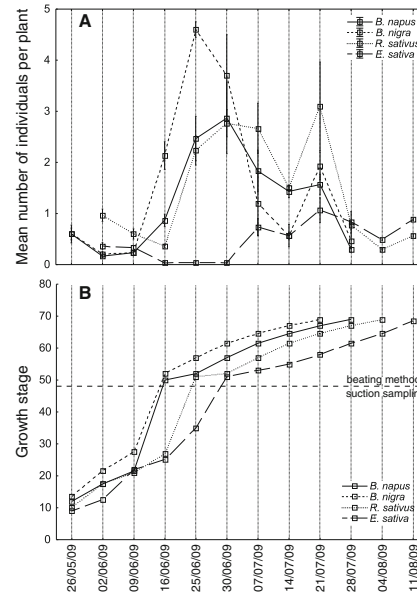

Fig. 1 **a** The mean (±SE) number of *Meligethes aeneus* adults per plot on *Brassica napus*, *B. nigra*, *Raphanus sativus* and *Eruca sativa* on different sampling dates in 2009 and **b** growth stages of these plants on those dates. Growth stages (Lancashire et al. 1991): *9* emergence, *10* cotyledons completely unfolded, *11–14* *1–4* leaves unfolded, *21* beginning of side shoot development, *21–27* *1–7* side shoots detectable, *27–29* end of side shoot development, *30–31* beginning of stem elongation, *31–39* stem elongation, *50* buds enclosed, *51–52* green buds, *53–55* bud extending, *55–59* yellow bud, *60–65*, 10–50 % flowering, *67* flowering declining, *68–69* end of flowering

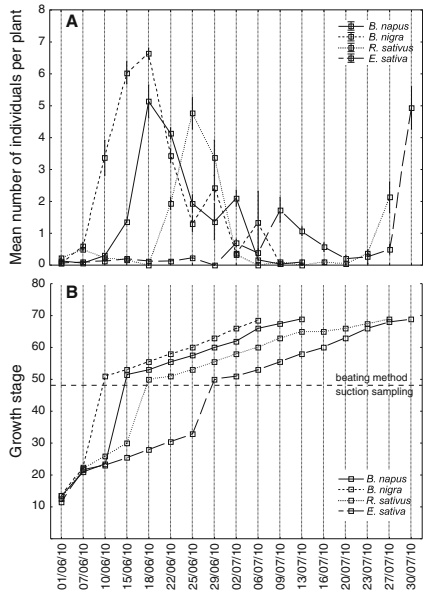

Fig. 2 **a** The mean (±SE) number of *Meligethes aeneus* adults per plot on *Brassica napus*, *B. nigra*, *Raphanus sativus* and *Eruca sativa* on different sampling dates in 2010 and **b** growth stages of these plants on those dates

Results

Abundance of overwintered pollen beetles

Adult overwintered pollen beetles started to colonize plants at their one to three leaf stage in both years (Figs. 1, 2). On the first sampling date (26 May) in 2009, the numbers of beetles on *B. nigra* and *B. napus* were similar (both species at GS 12–14), whereas *R. sativus* and *E. sativa* were unattractive to beetles, being still at the cotyledon stage (GS 9–10) (Fig. 1). After a week (2 June 2009), when all four plant species reached the first true leaf stage (GS 10–19), the numbers of beetles on *B. nigra* and *B. napus* decreased, while that on *R. sativus* increased and were significantly greater than on *B. nigra*, *B. napus* and *E. sativa* ($P < 0.05$). A similar distribution of beetles occurred also on 9 June 2009 at GS 21–27. On 16 June in 2009, more beetles were found on *B. nigra* than on *B. napus* (both species at GS 50–52), *R. sativus* and *E. sativa* (both at GS 23–27), and all differences between species were statistically significant (Fig. 1; $F_{(3,8)} = 20.36$, $P < 0.01$). By 25 June 2009, *B. nigra*, *B. napus* and *R. sativus* reached the most damage-susceptible stage for pollen beetles—green bud and green-yellow bud growth stages (GS 51–57)—and the number of beetles was significantly greater on *B. nigra* than on other species (Fig. 1; $F_{(3,8)} = 20.36$, $P < 0.01$). On 30 June 2009, *B. nigra* reached the flowering growth stage (GS 60) and all other plant species the green-yellow bud growth stage (GS 51–57). The number of beetles differed significantly between plant species ($F_{(3,8)} = 8.50$, $P < 0.01$). On 7 July 2009, both *B. nigra* and *B. napus* were at flowering growth stage (accordingly: GS 65 and 62), whereas *E. sativa* and *R. sativus* no open flowers yet and beetles were more evenly distributed (Fig. 1; $F_{(3,8)} = 3.39$, $P = 0.074$). A week later (14 July), the growth stages of plants were similar to those in the previous week, but the number of pollen beetles declined and the abundance of pollen beetles was significantly higher on *B. napus* and *R. sativus* than on *B. nigra* and *E. sativa* ($F_{(3,8)} = 15.47$, $P = 0.001$). On 21 July, only *E. sativa* no open flowers and the number of beetles was significantly greater on *R. sativus* than on *E. sativa* (LSD test, post hoc $P < 0.05$), but the overall distribution of beetles on different plant species was rather even ($F_{(3,8)} = 3.24$, $P > 0.05$).

In 2010, on 1 June, beetle numbers were similar on all plant species (GS 11–13; Fig. 2). By 7 June 2010, all plants had side branches (GS 20–23) and the numbers of beetles were greater on *B. nigra* and *R. sativus* than on *B. napus* and *E. sativa* (Fig. 2; $F_{(3,8)} = 14.11$, $P < 0.01$). On 15 June 2010, more beetles were found on *B. nigra*, followed by *B. napus* (both species at GS 50–52), *R. sativus* and *E. sativa* (both at GS 23–27) ($F_{(3,8)} = 268.13$, $P < 0.0001$) and numbers of beetles on *B. nigra* and *B. napus* differed significantly from *R. sativus* and *E. sativa* (LSD post hoc $P < 0.05$). By 18 June 2010, *B. nigra*, *B. napus* and *R. sativus* reached the most susceptible stage for pollen beetles—green bud and green-yellow bud growth stages (GS 51–57)—and the number of beetles was significantly greater on *B. nigra* than on other species including *B. napus* (Fig. 2; $F_{(3,8)} = 159.42$, $P < 0.0001$). By 22 June, the growth stages of plants had not notably developed further, but the number of beetles showed a substantial decline on *B. nigra* as well as on *B. napus*. At the same time, beetles started to gather on *R. sativus* where their number increased considerably, although there were significantly fewer beetles than on *B. nigra* and *B. napus* (Fig. 2; $F_{(3,8)} = 118.21$, $P < 0.0001$). On 25 June, pollen beetles clearly preferred *R. sativus*, where their number was significantly greater than on all other tested plants Fig. 2; $F_{(3,8)} = 42.01$, $P < 0.0001$). On 2 July 2010, *B. napus*, *B. nigra* and *R. sativus* reached the flowering stage and *E. sativa* was at the green-yellow bud stage and the overall number of pollen beetles decreased; however, their number was the greatest on *B. napus* (Fig. 2; $F_{(3,8)} = 30.12$, $P < 0.001$). On 9 July, *B. nigra* reached the

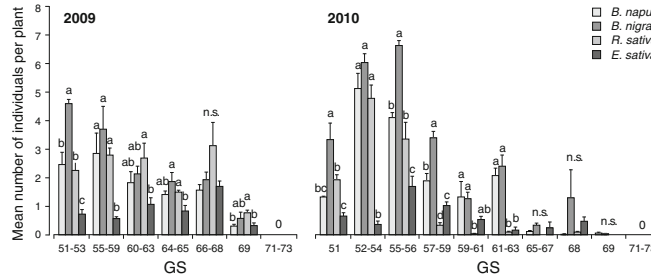

Fig. 3 The mean (±SE) abundance of *Meligethes aeneus* per plant (main raceme) at different growth stages (GS) of *Brassica napus*, *B. nigra*, *Raphanus sativus* and *Eruca sativa* in 2009 and 2010. *Different letters* indicate statistically significant differences between plants in the same GS group (LSD test; $P < 0.05$)

pod development stage and *B. napus* and *R. sativus* were at the flowering stage. The number of beetles was the greatest on *E. sativa* (the yellow bud growth stage) (Fig. 2; $F_{(3,8)} = 18.38$, $P < 0.001$).

In both years, over all sampling dates, the greatest number of beetles was found on *B. nigra* (2009: mean = 4.60 ± 0.15; 2010: mean = 6.63 ± 0.17). Overall, the mean numbers of beetles per plant during the GS 51–61 were: *B. napus* 2.01 ± 0.51 and 2.79 ± 0.72, *B. nigra* 3.38 ± 0.89 and 4.15 ± 1.07, *R. sativus* 2.29 ± 0.59 and 2.08 ± 0.54, and *E. sativa* 0.65 ± 0.17 and 0.89 ± 0.23 in 2009 and 2010, respectively.

To determine the importance of plant growth stage for colonization by pollen beetles, their abundance at different growth stages was analysed (Fig. 3). However, it is necessary to emphasize that the different plant species develop to different growth at different times. In the present analyses, this has not been taken into account, and only growth stages are compared. In 2009, at the green and yellow bud growth stage of plants (GS 51–53 and 55–59, respectively), the plant species had a significant influence on beetle abundance ($F_{(3,8)} = 31.6$; $P < 0.0001$ and $F_{(3,8)} = 5.97$; $P < 0.01$). There were more beetles on *B. nigra* than on other species (LSD post hoc $P < 0.05$). At the beginning of flowering (GS 60–63), plant species as a factor did not have a significant impact on the number of beetles ($F_{(3,8)} = 3.2$; $P = 0.07$). At full flowering (GS 64–65), plant species emerged as a determinant factor for beetle abundance again ($F_{(3,8)} = 4.58$; $P = 0.03$) and *E. sativa* was the least attractive. At the end of flowering (GS 66–68), beetle abundance was similar on all plant species (GS 66–68: $F_{(3,8)} = 2.1$; $P = 0.17$; GS 69: $F_{(3,8)} = 2.86$; $P = 0.1$).

In 2010, as in 2009, plant species had a significant impact on pollen beetle abundance at the green bud stage (GS 51; $F_{(3,8)} = 14.7$; $P = 0.001$) and *B. nigra* was the most attractive species (LSD post hoc $P < 0.001$; Fig. 3). At GS 52–54 (flower buds visible, but still closed), *E. sativa* had the fewest beetles (LSD post hoc $P < 0.05$) with no statistically significant differences between other species. This situation changed at bud extension (GS 55–56) and at yellow bud (GS 57–59) stages when significantly more beetles were found on *B. nigra* (LSD post hoc $P < 0.05$). The plant species had significant impact on the abundance of beetles in GS 55–56, 57–59, 59–61 and 61–63 (accordingly: $F_{(3,8)} = 32.15$, $P < 0.0001$; $F_{(3,8)} = 48.06$, $P < 0.001$; $F_{(3,8)} = 4.2$, $P = 0.05$ and $F_{(3,8)} = 26.9$, $P < 0.0001$). At the beginning of flowering (GS 61–63), significantly more beetles were found on *B. nigra* and *B. napus* than on *R. sativus* and *E. sativa*. From full flowering (GS 65) until the end of flowering (GS 69), plant species did not differ significantly in the number of beetles.

Abundance of pollen beetle eggs and larvae

Overall, the mean numbers of pollen beetle larvae per plot recorded by using the beating method in 2009 and 2010 differed significantly between plant species (Fig. 4a, 2009: ANOVA, $F_{(3,8)} = 8.64$, $P < 0.01$; Fig. 4b, 2010: $F_{(3,8)} = 27.67$, $P < 0.001$). In 2009, significantly more larvae were found on *B. nigra* than on other plant species (LSD post hoc $P \leq 0.05$); in 2010, there were significantly more larvae on *B. nigra* than on *B. napus*, *R. sativus* and *E. sativa* (LSD post hoc $P \leq 0.05$).

The mean numbers of pollen beetle eggs per bud and larvae per plot recorded by flower and bud dissection in 2009 differed significantly between plant species (eggs: Fig. 4c; $F_{(3,8)} = 18.82$, $P < 0.001$; larvae: Fig. 4d; $F_{(3,8)} = 11.93$, $P < 0.01$). The numbers of eggs per bud were significantly greater on *B. nigra* than on *B. napus*, *E. sativa* and *R. sativus* ($P < 0.01$, LSD post hoc). However, the buds from *E. sativa* were collected 5 days later than those from the other plant species and, therefore, concerning this plant species, the comparison here of *E. sativa* with other species may be overestimated. The

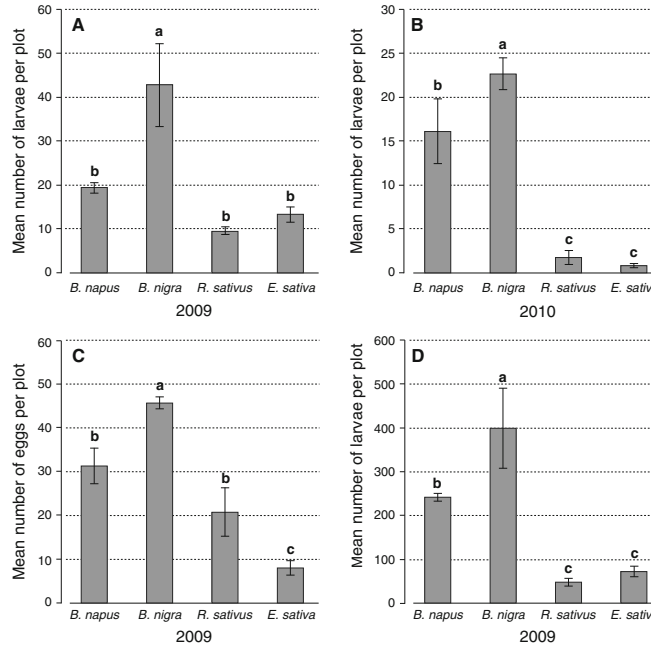

Fig. 4 Mean (±SE) number of *Meligethes aeneus* larvae counted from *Brassica napus*, *B. nigra*, *Raphanus sativus* and *Eruca sativa*, using beating of the main raceme (GS 50–69) in 2009 (**a**) and 2010 (**b**). **c** Mean (±SE) number of *M. aeneus* eggs per *plot* (counted from buds; $N = 75$) of different plant species (GS 51–52); and **d** mean number (±SE) of *M. aeneus* larvae per *plot* ($N = 30$) from the flowers of different plant species (GS 64–68) in 2009. *Different letters* indicate statistically significant differences between plants in 2009 and 2010 (LSD test; $P < 0.05$)

number of larvae per plant was significantly greater on *B. nigra* than on *B. napus*, *E. sativa* and *R. sativus* ($P < 0.05$; LSD post hoc). *Eruca sativa* and *R. sativus* both had low numbers of larvae, which did not differ significantly ($F_{(1,4)} = 3.26$, $P = 0.15$). Once again, there was a week between collection of samples from *B. napus* and *B. nigra* compared to *E. sativa* and *R. sativus*. Accordingly, we also compared *B. napus* and *B. nigra* separately from *E. sativa* and *R. sativus* and confirmed that *B. nigra* was preferred over *B. napus* for oviposition ($F_{(1,4)} = 8.45$, $P = 0.005$).

There were no significant differences in the number of buds and flowers per plot between *B. napus*, *B. nigra, E. sativa* and *R. sativus* ($F_{(3,8)} = 2.48$, $P = 0.14$); however, LSD post hoc test showed that *E. sativa* had significantly fewer buds and flowers than *B. nigra* ($P = 0.01$). Therefore, the comparison in the number of larvae in flowers of *E. sativa* may contain some underestimates, but comparison between other plant species is still correct.

Abundance of new-generation pollen beetles

Two plant species, *R. sativus* and *E. sativa,* were still flowering when the new generation of pollen beetles emerged from the soil. In both 2009 and 2010, more beetles were found on *E. sativa* than on *R. sativus* (ANOVA, 2009: $F_{(1,6)} = 4.87$, $P < 0.05$; 2010: $F_{(1,9)} = 12.41$, $P < 0.01$).

In 2009, the abundance of new-generation pollen beetles peaked, on both *E. sativa* and *R. sativus*, a week after their emergence from the soil (Fig. 5). Thereafter, their numbers declined steadily as they left for overwintering places during September. In 2010, new-generation beetles emerged from the soil earlier than in the previous year and left for their overwintering places approximately 2 weeks earlier, probably because the warmer summer hastened their development. In both years, the abundance of new generation of beetles followed a similar pattern on *R. sativus*, but differed on *E. sativa*. In 2010, the number of new-generation beetles on *E. sativa* was fairly constant from their emergence until mid-August, after which their numbers declined rapidly (Fig. 5).

Discussion

The relative attractiveness of *B.napus*, *B. nigra*, *E. sativa* and *R. sativus* for pollen beetle varied with plant growth

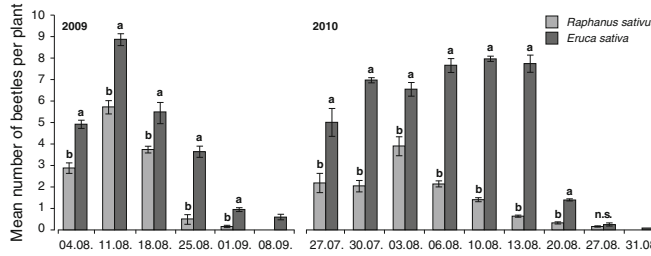

Fig. 5 Mean (±SE) number of new (summer)-generation adults of pollen beetles (side branches) per plant in *Eruca sativa* and *Raphanus sativus* in 2009 and 2010. *Different letters* indicate statistically significant differences between plants on the same date (ANOVA; $P < 0.05$)

stage and during the life cycle of the beetle. However, our data showed that *B. nigra* developed faster and attracted more adult beetles at the damage-susceptible stage (GS 51–61) than other tested plant species; therefore, it has potential as a trap crop in our climatic conditions. In addition, it was preferred for oviposition over other tested plant species as more eggs and larvae were found in the buds and flowers; thus, pollen beetles are not forced to migrate to oilseed rape for egg laying.

Pollen beetles started to colonize plants soon after their emergence from hibernation at plant establishment; the first were found sheltering on leaf axils, before stem extension. This is considerably earlier than reports in the literature, which suggest that they do not migrate to cruciferous crops until late stem extension (GS 39) (Petraitiene et al. 2008) or at "green bud" (GS 51) (Fritzsche 1957; Ruther and Thiemann 1997). In 2009, at the beginning of sampling at growth stages up to 25, the two *Brassica* species did not differ from each other in attractiveness, but the following year, *B. nigra* developed more rapidly than *B. napus* and was preferred by pollen beetles. The early colonizing beetles remained inactive for a week or more, hidden in the leaf axils. When flower buds appeared on the plants, they moved from the leaf axils onto the buds and were often seen inside them with only their abdomens protruding, presumably feeding. It is known that, after arriving in a cruciferous crop, a period of feeding time is needed for maturation of the ovaries of the females (Williams and Free 1978). At these early plant growth stages, the beetles were present in low numbers presumably because, on emergence from overwintering sites, they had a multiple choice of alternative flowering plants for feeding and only later concentrated on crucifers for mating and oviposition. Beetles did not move to *R. sativus* or to *E. sativa* when these reached the true leaf growth stage (GS 11) in either year, but stayed on the more advanced *Brassica* plant species. This concurs with other studies which show that pollen beetles within crops tend to be clustered (Free and Williams, 1978a; Ferguson et al. 2003) and that once the beetles have moved onto *Brassica* plants, they do not leave them (Borg and Ekbom 1996). However, we acknowledge that beetles' sampling differences between early and advanced growth stages may affect our results to a certain extent, but based on our experiences the data are comparable.

When plants reached the green bud stage (GS 50–53), the number of beetles increased on all tested plant species. Their abundance was similar on *B. napus*, *B. nigra* and *R. sativus*, being shifted chronologically accordingly to the growth stage of the plant species, but remained low on *E. sativa*. This concurs with what is known of their life cycle: pollen beetles generally infest oilseed crops at green bud, feed on pollen in the buds and flowers and lay their eggs in the buds (Williams and Free 1978; Cook et al. 2004, 2006b). In our study, *B. nigra* developed more rapidly than *B. napus*, flowering while *B. napus* was still in the bud stage, and became infested with the beetle to a greater extent. Thus, because of faster development, *B. nigra* lured adult beetles more than *B. napus* at GS 51 in both years, but when *B. napus* plants reached GS 53–55, the abundance of beetles did not differ between these plant species. *Eruca sativa* and *R. sativus* developed more slowly and were colonized by the beetle in large numbers later than the other tested species. Beetle abundance increased steadily after the green bud stage on all tested species; they were most numerous at bud extension and yellow bud stages and thereafter generally declined. Petraitiene et al. (2008) similarly reported that spring oilseed rape colonization by the beetle was most intensive at the yellow bud growth stage.

The crucifer species tested were all attractive to the overwintered generation of pollen beetle, with the exception of *E. sativa*, on which the population remained relatively low during colonization. Ekbom (1998) also showed *E. sativa* to be relatively unattractive to the beetle. However, more beetles were recorded on *B. nigra* than on *B. napus*.

Plant growth stage is known to influence the spatial distribution of the pollen beetle (Free & Williams 1978b; Ferguson et al. 2003; Frearson et al. 2005). Therefore, to effectively protect the main crop, the attractiveness of plants at different growth stages must be taken into consideration when choosing trap crops and planting time. Our data showed that at the most damage-susceptible growth stage of *B. napus* (GS 51–59), beetles clearly preferred *B. nigra* over all other species in both years (Fig. 3). However, as has been stressed, differences in growth stages do not reflect differences in the time factor. Nevertheless, the comparison of attractiveness of different growth stages provides us with data on how to manipulate planting/seeding dates. Hence, seeding of *B. nigra* a week before the main crop may lead to reduced infestation of the latter.

Brassica nigra was preferred for oviposition by the pollen beetle over *B. napus*, *E. sativa* and *R. sativus*; more eggs as well as more larvae were recorded on *B. nigra* than on the other three species according to both bud dissection and beating analysis. This contrasts with the results of Ekbom and Borg (1996) who found significantly fewer larvae on *B. nigra* than on spring *B. napus*. One explanation for this difference may be that our results were from the field, whereas Ekbom and Borg (1996) carried out their study in the laboratory. Ulber and Thieme (2007) also reported that the numbers of eggs laid on *B. nigra,* as well as on *Sinapis alba* L. (white mustard) and *Crambe abyssinica* Hochst. ex R.E.Fries (crambe), were lower than on *B. napus*, *Brassica campestris* L. (syn. *rapa*) and *Brassica juncea* (L.) (trowse mustard). However, because of different development of plants, the sampling to assess larval abundance in flowers from *B. napus* and *B. nigra* was carried out a week earlier than the other two plant species. Regarding the egg analyses, buds from *E. sativa* were sampled 5 days later than from other plant species. This may influence our results to a certain extent.

In our experiment, significantly more eggs per bud were found on *R. sativus* than on *E. sativa*, but the number of larvae in flowers of these plant species did not differ. Few larvae were found in *R. sativus* and *E. sativa* even though both species were colonized. In our study, buds form *E. sativa* were sampled 5 days later than *R. sativus*, but in our case this confirms the results because beetles had a longer time for oviposition in *E. sativa* and still there were fewer eggs. The number of larvae in the flowers of *R. sativus* was substantially fewer than the number of eggs in the buds indicating that this plant species may act as a 'dead-end trap crop' (Shelton and Nault 2004), which can lure pollen beetles for egg laying but does not offer suitable food for development of the larvae. Few eggs were laid on *E. sativa* and this species may be therefore used primarily as a food plant for pollen beetle. Other cruciferous species, e.g. *S. alba* and *C. abyssinica*, have similarly been reported to be primarily food plants and little used for oviposition (Winfield 1961; Ekbom and Borg 1996). Plant growth stage greatly influences plant attractiveness to the beetle. The abundance of buds for oviposition is an important determinant of residence time on plants (Nilsson 1988; Frearson et al. 2005) and so will affect population density on different species. In our conditions, *E. sativa* flowered later than the other species, so that the main periods of immigration and oviposition of the beetles were not well synchronized with the growth stages of the plant suitable for feeding and egg laying. Further, the size and the oblong shape of the buds in *E. sativa* may have deterred oviposition in this species (Ekbom 1998).

New-generation pollen beetles emerge from pupation in the soil in late July and August; the beetles then disperse to feed on flowering plants of different families to accumulate fat reserves for the winter before leaving to find overwintering sites (Williams and Free 1978). Food intake is important for this generation and has a strong impact on their winter survival (Hokkanen 1993; Hiiesaar et al. 2011). Our experiment demonstrated that, at this time, the beetles found acceptable food on the late-flowering *E. sativa* and *R. sativus* plants, which produce multiple secondary flowering stems; they were more abundant on *E. sativa* than on *R. sativus*, perhaps because the yellow flowers of the former were preferred over the pale violet-coloured flowers of the latter (Cook et al. 2006a). Several studies have demonstrated that new-generation beetles are attracted by both flower odour and colour, especially yellow (Charpentier 1985; Jönsson 2005; Cook et al. 2006b; Jönsson et al. 2007). However, flowers of other colours are also accepted and we have observed new-generation adults in abundance on both the yellow-coloured flowers of *Sonchus arvensis* L. (sowthistle) and the purple disk flowers of *Cirsium arvense* (L.) (thistle) (Hiiesaar et al. 2011). Colour cues have been reported to be relatively more important than olfactory cues to new-generation beetles seeking food plants than to overwintered generation beetles seeking cruciferous plants for oviposition (Jönsson 2005).

Cook et al. (2006b, 2007b) have shown that spring turnip rape/spring oilseed rape can successfully protect oilseed rape from pollen beetles and is an excellent trap crop plant for oilseed rape. Our results suggest that *B. nigra* has similar features; of the three species tested in our experiment, *B. nigra* has the most potential as a trap crop plant to protect spring oilseed rape during its vulnerable bud stage, due to its faster development and acceptability both for food and oviposition by overwintered pollen beetle adults. Although *R. sativus* was attractive during colonization and for oviposition, few larvae were found in the flowers, suggesting a high mortality rate of eggs and larvae in its flowers. Therefore, growing of mixture of *B. nigra*

and *R. sativus* may have the dual potential of trap- and 'dead-end' trap crop, luring pollen beetles away from oilseed rape and preventing the development of the eggs laid. Further work is required to test the feasibility and effectiveness of this strategy. Furthermore, use of a late-flowering crop, like *E. sativa* and *R. sativus,* could expand the potential effectiveness of a trap crop system by attracting new-generation beetles at the end of the growing season. Both of these species have side branches in flower when new-generation beetles need food before seeking overwintering sites. Such a trap could give early warning of the emergence and abundance of new-generation beetles, and would minimize the local build-up of the population. If these plant species were grown as follow-on crops (cover crops), they could be ploughed in during August, when the new generation of pollen beetles feed on the flowers. Destruction of the crop at this time would also kill a large portion of beetles and remove them from the local agroecosystem. Moreover, *R. sativus* and *E. sativa* would provide other benefits to the crop production system. They are both biofumigants, reducing the population of nematode pests, weeds and diseases (Ngouajio et al. 2004). In addition, *R. sativus* has low production costs (Domingos et al. 2008). This characteristic, as well as its great ability to recycle nutrients such as phosphorus and nitrogen, makes it one of the most commonly used species for green fertilization during the interval between harvests of other crops. Additionally, *E. sativa* interferes with development and reproduction of the northern root-knot nematode, *Meloidogyne hapla*, and thus has potential as a trap crop to control *M. hapla.* It is used as a green manure trap crop primarily to reduce populations of sugar beet cyst nematode *Heterodera schachtii* (Lehrsch and Gallian 2010).

Further study of the relationships between these plant species and pollen beetles in the field is desirable. Manipulation of sowing dates so that all tested plant species reach the same growth stages concurrently would give a better comparison of host plant preferences by the beetles.

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IV

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Fatal attractiveness – host plant may kill its pest's larvae.

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FATAL ATTRACTIVENESS – HOST PLANT MAY KILL ITS PEST'S LARVAE

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Keywords: *Meligethes aeneus*, dead-end trap crop, *Brassica napus*, *B. rapa*, *B. juncea*, *B. nigra*, *Sinapis alba*, *Raphanus sativus, Eruca sativa*

Running title: Fatal attractiveness

Abstract. In sustainable pest management, insect pests can be manipulated by utilising their relative attractiveness to different host plants. Plants attractive for oviposition but not offering a suitable environment for development are called dead-end trap crops. In this study, the oviposition activity and larval survival of *Meligethes aeneus* (Fab.) in the buds of *Brassica napus* L., *B. rapa* L., *B. nigra* L., *B. juncea* L., *Eruca sativa* Mill., *Raphanus sativus* Pers. and *Sinapis alba* L. were compared. Overall infestation rate of buds varied from 0 to 71%, the least attractive plants were *S. alba* and *E. sativa*. Egg clutch size per bud was greater on *B. nigra* and lower on *S. alba* and *E. sativa* than on *B. napus*. Dead larvae were found in *E. sativa* and *R. sativus* buds. In conclusion, *M. aeneus* preferred to oviposit on Brassica species than on cruciferous plants of the other genera and *R. sativus* has potential as a dead-end trap-crop because a third of the larvae failed to survive.

Introduction

Pollen beetle, *Meligethes aeneus* Fab. (Coleoptera, Nitidulidae) is the most important pest of oilseed crops in Europe (Williams 2010). Adult beetles emerge from their overwintering sites in spring when temperature exceeds 8–10 °C and feed on pollen from different flowering plants. After maturation feeding, they oviposit only in the flower buds of brassicaceous plants (Free and Williams 1978; Metspalu et al. 2011). Feeding and oviposition damage by adults and first instar larvae within the bud can result in bud abscission and loss of yield. Second instar larvae do not cause economic yield loss, because they feed on pollen from open flowers (Williams and Free 1978). Therefore, only plants at the green and yellow bud growth stages of oilseed rape are susceptible to yield diminishing damage (Axelsen and Nielsen 1990; Cook and Denholm 2008; Tatchell 1983). Control of pollen beetle relies mainly on synthetic pesticides which may not decrease the pest population (Hokkanen 2000), and can even increase it (Veromann et al. 2008); it also leads to development of pyrethroid resistance in pollen beetles (Thieme et al. 2010). Therefore, the development of environmentally safe sustainable control methods for the pollen beetle is a key issue in oilseed rape production.

Behavioural manipulation of insect pests (through their host plant preferences) is a useful tool in ecologically sustainable and economically viable integrated crop management. There is a growing interest in the value of enhanced agro-ecosystem diversity and "push-pull" strategies for suppression of insect pests. One ('pull') component of this strategy is trap-cropping which is based on the different attractiveness of host plants for pests (Cook et al. 2007a). Trap cropping can reduce reliance on insecticide applications and it has been shown to have a potential to control several cruciferous pests, including *M. aeneus* (Bartlet et al. 2004; Blight and Smart 1999; Cook et al. 2006, 2007a,b, 2013; Frearson et al. 2005; Hokkanen 1991; Veromann et al. 2012; Vinatier et al. 2012). Trap crops can consist of one or several host plant species for a particular pest, grown to lure pest species away from the main cash crop and thereby limit pest damage to the main crop (Cook et al. 2007a; Hokkanen 1991; Shelton and Badenes-Perez 2006). In addition, application of a trap crop strategy in oilseed rape can enhance the presence and diversity of parasitoid species in agro-ecosystems (Kaasik et al. 2013, 2014; Kovács et al. 2013), to efficiently control the pest population (Ulber et al. 2006; Veromann et al. 2011). Trap crops are classified as either conventional trap crops, which are more attractive than the main crop for pest feeding and/or oviposition, or dead-end trap crops, which, although being highly attractive to insects, do not support their development. Another important requirement for both conventional and dead-end trap cropping is that the trap crop has to be highly preferred by the pest (Hokkanen 1991; Shelton and Nault 2004). Dead-end trap crops could be obtained either via the intrinsic characteristics of a plant species (Badenes-Perez et al. 2005), such as nutritional inadequacy, through its chemical defence properties (Shelton and Nault 2004; Shelton and Badenes-Perez 2006), or created by introduction of an insecticidal transgene, such as a *Bacillus thuringiensis* Berliner gene (Cao et al. 2005). One well-known example is yellow rocket, *Barbarea vulgaris* var. *arcuata* R. Br. which acts as a dead-end trap crop for the diamondback moth, *Plutella xylostella* (L.) via its intrinsic characteristics (Lu et al. 2004; Shelton and Nault 2004, 2006). Caterpillars cannot complete development on this plant; this inability to survive has been attributed to the feeding deterrent, monodesmosidic triterpenoid saponin (Shinoda et al. 2002). Results from laboratory and greenhouse studies showed that vetiver grass (*Vetiveria zizanioides* (L.) Nash) is highly preferred for oviposition by the spotted stem borer (*Chilo partellus* (Swinhoe)) but larval survival is extremely low (Van den Berg et al. 2003). Rucola (*Eruca sativa* L.) has been shown to be an effective dead-end trap crop for root-knot nematodes, *Meloidogyne* spp. (Melakeberhan et al. 2006) and stinkweed, *Thlaspi arvense* L. as a dead-end trap crop for the cabbage looper (*Trichoplusia ni* Hübner) (Cameron et al. 2007).

Furthermore, the host plant's quality is essential because it influences the development and fecundity of insect pests (Awmack and Leather 2002; Kehat and Wyndham 1972). For normal growth and development of larvae the proportions of nutritional elements in the food plant are of primary importance (Awmack and Leather 2002), and host plant quality influences herbivore insect survival and development time (Metspalu et al. 2013). In addition, several different factors that are relevant for plant growth also have an impact on the development of insects. Foliar nitrogen concentration is often cited as one of the main factors limiting the population growth of insect herbivores (Mattson 1980; Tao et al. 2013) and it is known to affect the relative growth rate, development time and survival of a range of phytophagous species (Awmack and Leather 2002; Staley et al. 2009) by increasing plant toxicity (Tao et al. 2013). Veromann et al. (2013) have shown that different nitrogen fertilization levels of plants can have an effect on the behaviour of *M. aeneus*. Hopkins and Ekbom (1999) have shown that *M. aeneus* is capable of changing oviposition rate to match resources available. Studies of the interactions between pollen beetles and potential trap crop plants in field conditions are scarce and results are in close dependency on the study area therefore

are sometimes controversial (Cook et al. 2007b; Hokkanen 1991; Kaasik et al. 2013, 2014; Veromann et al. 2012). Moreover, the only study that hypothesized about dead-end trap crops that focus on pollen beetles is that by Veromann et al. (2012). Therefore, this study was conducted to continue the aforementioned study to find out whether these assumptions about probable dead-end traps crops for pollen beetles could be veritable.

The principal focus of this study was to compare *M. aeneus* oviposition preference and larval survival on the buds of *Brassica napus*, *B. rapa*, *B. juncea*, *B. nigra*, *Sinapis alba*, *Raphanus sativus* and *Eruca sativa*, to find a potential conventional and dead-end trap-crop for oilseed rape.

Materials and methods

Study area and experimental design

Studies were carried out in an experimental field of the Estonian University of Life Sciences in 2011 and 2012, Tartu County (58°21' latitude L26°39' longitude). The plants were grown in a randomized complete block design with three replicates of each of the following species: *B. napus* (cv. 'Maskot'), *B. rapa*, *B. juncea*, *B. nigra*, *Eruca sativa* (cv. 'Poker'), *Raphanus sativus* (cv. 'Bille') and *Sinapis alba* (cv 'Braco'). In 2011, seeds were obtained from the seed bank of the Estonian University of Life Sciences. In 2012, seeds collected in the autumn from plants in the 2011 experiment were used. In both years, each plot was 1x5m with a 1m wide buffer zone of bare soil around it to minimize inter-plot interactions. The whole experimental field was surrounded by red clover. Plots were sown concurrently on 9 May 2011 and 15 May 2012, at 250 seeds per m^2. Neither fertilizers nor pesticides were used. Plant growth stage (BBCH) was assessed using the decimal code system of Meier (2001).

Insect sampling

To determine the oviposition activity of *M. aeneus*, 50 green (BBCH 51–53) and 50 yellow (BBCH 58–59) buds were collected from randomly chosen plants on each plot (Table 1). In the laboratory, all buds were dissected under the microscope (Olympus SZ-CTV) on the day of collection; the numbers of infested and non-infested buds and the numbers of eggs and larvae (live and dead) per infested bud were recorded.

Table 1. Sampling dates of green (BBCH 51–53) and yellow (BBCH 59) buds from tested plant species in 2011 and 2012.

	Green buds		Yellow buds	
	2011	2012	2011	2012
Brassica napus	14 June	28 June	17 June	3 July
Brassica rapa	14 June	28 June	17 June	3 July
Brassica juncea	14 June	28 June	17 June	3 July
Brassica nigra	14 June	28 June	17 June	3 July
Sinapis alba	14 June	28 June	17 June	3 July
Raphanus sativus	14 June	28 June	17 June	3 July
Eruca sativa	14 June	28 June	17 June	3 July

Statistical analyses

The statistical significance of plant species and year on the number of beetle eggs and larvae in green and yellow buds as well as the number of live and dead larvae were calculated by using Wald statistic Type III empirical standard error analysis with the Poisson model and

log link function considering the fixed effects of year and plant species. The response variable was infested bud. Differences of means between plant species were made using the GENMOD procedure Differences of Least Squares Means test. Comparisons of infested green and yellow buds of different plant species and years were made with also by Wald statistic Type III empirical standard error analysis but with Binomial distribution and *logit* link function. The analyses were conducted with SAS 9.1 software (SAS Institute, Inc., Cary, NC, USA). All results were considered statistically significant at $P \leq 0.05$.

Results

Damage to buds

Plant species had a significant impact on the infestation rate of green (2011: $\chi^2 = 58.20$, df = 6, $P < 0.0001$; 2012: $\chi^2 = 124.41$, df =6, $P < 0.0001$) and yellow buds (2011: $\chi^2 = 80.25$, df = 6, $P < 0.0001$; 2012: $\chi^2 = 133.70$, df = 6, $P < 0.0001$) by pollen beetle eggs and larvae each study year as well as over the two-year study period (green buds: $\chi^2 = 51.90$, df = 6, $P < 0.0001$; yellow buds: $\chi^2 = 78.76$, df = 6, $P < 0.0001$). The infestation rate was similar in both study years at both growth stages since year as a factor had no influence (green: $\chi^2 = 2.35$, df = 1, $P = 0.13$; yellow: $\chi^2 = 1.98$, df = 1, $P = 0.16$).

In 2011, the greatest number of infested green buds per plot was on *B. nigra*, significantly more than on *B. rapa* ($\chi^2 = 3.75$, df = 1, $P = 0.05$) and *S. alba* ($\chi^2 = 32.10$, df = 1, $P < 0.0001$). The same tendency continued at the yellow bud growth stage of plants with the greatest number of infested buds per plot on *B. nigra* but, at this time, it differed significantly from *B. juncea* ($\chi^2 = 6.98$, df = 1, $P < 0.01$), *S. alba* ($\chi^2 = 23.38$, df = 1, $P < 0.0001$) and *E. sativa* ($\chi^2 = 53.95$, df = 1, $P < 0.0001$). In both years, as well as in both growth stages, the lowest numbers of infested buds were on *S. alba* and *E. sativa* (in 2011, at green bud stage there were no infested buds on *E. sativa,* which differed significantly from all other plant species (see Table 2 for full statistical analyses)).

In 2012, the greatest number of infested green buds per plot was on *B. rapa*, significantly more than on the other species (*B. napus*: $\chi^2 = 7.43$, df = 1, $P < 0.01$; *B. juncea*: $\chi^2 = 10.79$, df = 1, $P < 0.001$; *B. nigra*: $\chi^2 = 6.83$, df = 1, $P < 0.01$; *R. sativus*: $\chi^2 = 11.53$, df = 1, $P < 0.001$; *S. alba*: $\chi^2 = 58.57$, df = 1, $P < 0.0001$; *E. sativa*: $\chi^2 = 84.66$, df = 1, $P < 0.0001$). As in 2011, the least infested plants at green bud stage were *S. alba* and *E. sativa* which differed

Table 2. Mean (±SE) number of infested green and yellow buds per plot (N=50) in different plant species in two growing seasons.

	Number of infested green buds			Number of infested yellow buds		
	Year			Year		
Plant species	2011	2012	Mean	2011	2012	Mean
Brassica napus	0.64 ± 0.04ab	0.54 ± 0.04a	0.59 ± 0.03a	0.65 ± 0.04ab	0.71 ± 0.04a	0.68 ± 0.03a
Brassica rapa	0.60 ± 0.04a	0.70 ± 0.04b	0.61 ± 0.03a	0.65 ± 0.04ab	0.64 ± 0.04ab	0.65 ± 0.03a
Brassica juncea	0.65 ± 0.04ab	0.51 ± 0.04a	0.58 ± 0.03a	0.57 ± 0.04a	0.63 ± 0.04ab	0.60 ± 0.03a
Brassica nigra	0.71 ± 0.04b	0.55 ± 0.04a	0.63 ± 0.03a	0.72 ± 0.04b	0.64 ± 0.04ab	0.68 ± 0.03a
Raphanus sativus	0.64 ± 0.04a	0.51 ± 0.04a	0.57 ± 0.03a	0.64 ± 0.04ab	0.55 ± 0.04b	0.59 ± 0.03a
Sinapis alba	0.31 ± 0.04c	0.24 ± 0.03c	0.27 ± 0.03b	0.44 ± 0.04c	0.26 ± 0.04c	0.64 ± 0.03b
Eruca sativa	0.00 ± 0.00*	0.05 ± 0.02d	0.03 ± 0.03c	0.28 ± 0.04d	0.20 ± 0.03c	0.24 ± 0.03b

Different letters indicate significant differences between plant species within the given year ($P<0.05$ according to GENMOD Differences of Least Squares Means test)

* The analysis gave no data to compare the mean of *E. sativa*.

significantly from all other plant species and also from each other (χ^2 = 17.62, df = 1, P < 0.0001). At the yellow bud stage, the infestation rate of buds on *B. napus*, *B. rapa*, *B. juncea* and *B. nigra* was adjusted and significantly more infested buds were on *B. napus* than on *R. sativus* (χ^2 = 8.82, df = 1, P < 0.01), *S. alba* (χ^2 = 56.98, df = 1, P < 0.0001) and *E. sativa* (χ^2 = 71.10, df = 1, P < 0.0001) only (Table 2).

When data were pooled, the infestation rate of green and yellow buds was similar on *B. napus, B. rapa, B. juncea, B. nigra* and *R. sativus,* with significantly fewer on *S. alba* and *E. sativa* (Table 2).

Cluch size

To estimate pollen beetle oviposition activity on different plant species, we compared the number of eggs and larvae per infested bud. Plant species had a significant influence on the number of eggs in green buds (χ^2 = 93.57, df = 1, P < 0.0001) as well as in yellow buds (χ^2 = 202.34, df = 1, P < 0.0001) over the two-year study period. The year as a factor had no influence on the number of larvae at the green bud stage of plants (χ^2 = 0.09, df = 1, P = 0.76) but had an impact on these numbers at the yellow bud stage of plants (χ^2 = 9.93, df = 1, P = 0.0016).

In 2011, significantly more eggs and larvae were found in green as well as in yellow buds on *B. nigra* than all other plant species (In green buds: *B. napus*: χ^2 = 13.90, df = 1, P = 0.0002; *B. rapa*: χ^2 = 5.77, df = 1, P = 0.016; *B. juncea*: χ^2 = 14.04, df = 1, P = 0.0002; *R. sativus*: χ^2 = 71.04, df = 1, P < 0.0001; *S. alba*: χ^2 = 73.86, df = 1, P < 0.0001. In yellow buds: *B. napus*: χ^2 = 7.03, df = 1, P = 0.0080; *B. rapa*: χ^2 = 5.33, df = 1, P = 0.021; *B. juncea*: χ^2 = 22.14, df = 1, P < 0.0001; *E. sativa*: χ^2 = 110.87, df = 1, P < 0.0001; *R. sativus*: χ^2 = 122.81, df = 1, P < 0.0001; *S. alba*: χ^2 = 162.28, df = 1, P < 0.0001). Beetles laid eggs into the green buds in similar numbers on *B. napus*, *B. rapa* and *B. juncea*, significantly fewer eggs were found from *R. sativus* and *S. alba* than all other plant species (See Table 3 for full statistical analyses). At yellow bud stage, the number of larvae found on *B. napus* and *B. rapa* was similar (χ^2 = 0.11, df = 1, P = 0.74), fewer than on *B. nigra* but greater than on *R. sativus*, *S. alba* and *E. sativa* (Table 3).

In 2012, the greatest number of eggs in green buds were on *R. sativus* which differed significantly from *B. rapa* (χ^2 = 10.82, df = 1, P < 0.001), *B. juncea* (χ^2 = 6.78, df = 1, P = 0.0092), *B. nigra* (χ^2 = 7.64, df = 1, P = 0.0057), *S. alba* (χ^2 = 36.33, df = 1, P < 0.0001)

Table 3. Mean (±SE) number of eggs and larvae per infested green and yellow bud in different plant species in two growing seasons.

	Number of eggs/larvae in green buds			Number of larvae in yellow buds		
	Year			Year		
Plant species	2011	2012	Mean	2011	2012	Mean
Brassica napus	1.98 ± 0.05a	2.07 ± 0.05ab	2.02 ± 0.04a	2.31 ± 0.04a	2.08 ± 0.05ad	2.18 ± 0.03a
Brassica rapa	2.16 ± 0.05a	1.82 ± 0.05a	1.98 ± 0.04a	2.35 ± 0.04a	2.52 ± 0.05bc	2.43 ± 0.03bc
Brassica juncea	1.98 ± 0.05a	1.88 ± 0.06a	1.94 ± 0.04ac	2.05 ± 0.04b	2.57 ± 0.05c	2.31 ± 0.03abc
Brassica nigra	2.51 ± 0.04b	1.87 ± 0.05a	2.23 ± 0.03b	2.63 ± 0.03c	2.13 ± 0.06a	2.40 ± 0.03c
Raphanus sativus	1.38 ± 0.06c	2.29 ± 0.05b	1.78 ± 0.04c	1.84 ± 0.05d	2.38 ± 0.06acd	1.84 ± 0.04d
Sinapis alba	1.0 ± 0.10d	1.14 ± 0.04c	1.06 ± 0.07d	1.00 ± 0.07e	1.46 ± 0.11e	1.18 ± 0.06e
Eruca sativa	0.0 ± 0.0*	1.13 ± 0.22c	1.06 ± 0.23d	1.00 ± 0.09e	1.20 ± 0.13e	1.09 ± 0.08e

Different letters indicate significant differences between plant species within the given year (P<0.05 according to GENMOD Differences of Least Squares Means test)

* The analysis gave no data to compare the mean of *E. sativa*.

and *E. sativa* (χ^2 = 9.70, df = 1, P = 0.0018) but had similar number of eggs as *B. napus* (χ^2 = 1.90, df = 1, P = 0.17; Table 3). The egg laying activity of these beetles changed from green to yellow bud stage since the highest mean numbers of eggs per bud were found on *B. juncea*, *B. rapa* and *R. sativus* (see Table 3 for detailed significant differences).

When data were pooled over the years, the most preferred plant species for oviposition during green bud stage was *B. nigra* which differed significantly from all other plant species (*B. napus*: χ^2 = 3.71, df = 1, P = 0.05; *B. rapa*: χ^2 = 5.91, df = 1, P = 0.015; *B. juncea*: χ^2 = 7.55, df = 1, P = 0.006; *E. sativa*: χ^2 = 8.29, df = 1, P < 0.004; *R. sativus*: χ^2 = 18.46, df = 1, P < 0.0001; *S. alba*: χ^2 = 81.77, df = 1, P < 0.0001). Similar numbers of eggs were found on *B. napus*, *B. rapa* and *B. juncea*. Fewest eggs per bud were on *S. alba* and *E. sativa* (Table 3). Over the study period, the mean number of larvae per yellow bud was similar on *B. rapa*, *B. juncea* and *B. nigra* but significantly fewer larvae were found on *B. napus* than on *B. rapa* (χ^2 = 5.28, df = 1, P = 0.022) and *B. nigra* (χ^2 = 4.00, df = 1, P = 0.045). The mean number of larvae in yellow buds of *R. sativus* was significantly fewer than all other species but *S. alba* and *E. sativa* which had the fewest larvae (Table 3).

Dead-end plants

To estimate the suitability of the host plant for the development of beetles' larvae, dead larvae from all buds were counted. Dead larvae were found only in the yellow buds of *R. sativus* and *E. sativa*. The plant species had a significant effect on the mean number of live as well as dead larvae (accordingly: χ^2 = 8.89, df = 1, P = 0.029; χ^2 = 22.58, df = 1, P < 0.0001) but year as a factor had a significant influence only on live larvae (χ^2 = 71.40, df = 1, P < 0.0001). The numbers of live and dead larvae were always greater on *R. sativus* except in 2011 when live larvae were found in similar numbers in both plant species (live larvae: 2011: χ^2 = 0.01 df = 1, P = 0.92; 2012: χ^2 = 18.59, df = 1, P < 0.0001; dead larvae: 2011: χ^2 = 11.60, df = 1, P < 0.001; 2012: χ^2 = 10.74, df = 1, P = 0.001; Fig. 1). On *R. sativus*, mortality reached 45.5% in 2011, but, the following year decreased to only 26.7%. Over the two study years, 35% of larvae on *R. sativus* were dead. On *E. sativa*, mortality of larvae was half that on *R. sativus* with 23.8% in 2011, 13.9% in 2012 and over both years 19.3%.

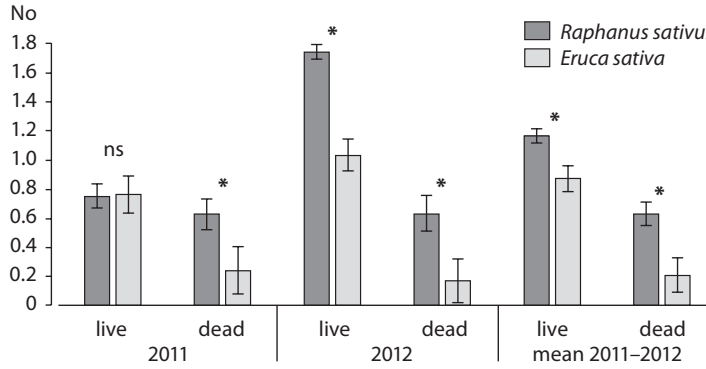

Figure 1. Mean (±SE) number of live and dead larvae of *Meligethes aeneus* in infested yellow buds per plot on different host plant species. * indicates significant differences between plant species (P<0.05 according to GENMOD Differences of Least Squares Means test), ns – not significant.

Discussion

Dead-end plants

This study shows, for the first time, that *R. sativus*, and to some extent also *E. sativa*, can perform as dead-end trap-crop plants for *Meligethes aeneus* larvae. Female pollen beetles oviposited equally on *R. sativus*, *B. napus, B. rapa, B. juncea* and *B. nigra* but larval survival was significantly lower on *R. sativus* than on the other plant species. This confirms the view of Veromann et al. (2012), who suggested that *R. sativus* may act as a dead-end trap crop as they found more eggs in its buds than larvae in its flowers. In 2011, the ratio of dead to live larvae on *R. sativus* was almost equal, whereas in 2012, more than a quarter were dead so that when the data were pooled over two years the mortality rate was 35%. The difference in mortality rates in different years could be caused by several factors, e.g. differences in the rate of growth of plants caused by weather conditions, temperature, humidity rates etc., which can change food plant quality. The attractiveness of *E. sativa* and *S. alba* to *M. aeneus* for oviposition was rather low as the average infestation rate stayed below 25%. Although, dead larvae were also found on *E. sativa*, the average mortality of larvae was much lower than on *R. sativus* at around 20%. A relatively large proportion of the observed mortality on *R. sativus* was associated with the larval moulting process. The larvae in buds died before shedding their old cuticles; presumably the epidermal cells failed to separate from the old cuticle. Such effects are thought to be caused by disruptions of the complex interactions between hormones that stimulate the moulting process. The main hormones involved in growth regulation in insects are ecdysone and 20-hydroxy-ecdysone (moulting hormones) and juvenile hormones (Hiruma and Riddiford 2001). The balanced interaction of these hormones ensures normal insect development (Nijhout 1994). Moulting requires a balance of hormones and other physiological changes to be successful, and can occur only when the insect has reached a certain minimum body mass threshold for the growth stage (Davidowitz et al. 2003). The growth and moulting processes are influenced by several factors, the most important being food availability and quality. After that, the type of food consumed, the quantity, and the efficiency with which it is used by the insect determine the availability of nutrients for growth during development (Browne and Raubenheimer 2003). In the present study, the lethal larval moulting observed was most likely caused by interference with hormone production/release by toxic compounds in tissues of *R. sativus* as well as low quality of food. Both factors could cause histopathological changes in the endocrine system of the larvae, and disorders in the secretion of hormones. The larvae died in the green buds of *E. sativa* shortly after hatching which suggests that the quality of this plant species was too low for their development. However, the plant may also contain feeding inhibitors or components toxic to the larvae. For instance, Shinoda et al. (2002) found that a cruciferous plant, yellow rocket (*B. vulgaris* L.), contained the feeding deterrent triterpenoid saponin which killed all *P. xylostella* larvae in their first growth stage. In addition, shepherd's purse, *Capsella bursa-pastoris* (L.) also contains saponins which deter feeding of *Phyllotreta* spp. (Nielsen 1989) and *P. chrysocephala* L. (Bartlet and Williams 1991; Metspalu et al. 2011). Furthermore, Nilsson (1988) supposed, that the failure of pollen beetle larvae to mature is dependent on bud abortion of oilseed rape and the relationship between the rates of larval and host plant development. Thus interpretation can be complicated because multiple mechanisms may operate simultaneously.

Damage to buds

Our results showed that *M. aeneus* differentiated between plant species for oviposition. The degree of damage to buds of different plant species varied from 0 to 71% and was quite similar between years. In this study, only small differences in the number of infested buds were

found between Brassica species. Female beetles preferred *B. nigra* (65%), *B. napus* (63%) and *B. rapa* (63%), followed by *B. juncea* (59%) and *R. sativus* (58%). These equally acceptable Brassica species for pollen beetles are closely related and have similar morphology. The least attractive plants for oviposition were *S. alba* (26%) and *E. sativa* (13%), species which differ from the Brassica species in morphology (*E. sativa*) as well as in chemistry (*S. alba*) (Tollsten and Bergstrom 1988; Ekbom 1998). The relative unattractiveness of *S. alba* and *E. sativa* for oviposition of pollen beetles has been shown also by Borg and Ekbom (1996), Ekbom (1998), Veromann et al. (2012) and Kaasik et al. (2013, 2014). Hopkins and Ekbom (1996) concluded that one of the reasons for this kind of choice is low host quality that does not increase the physiological motivation of the pollen beetle to oviposit. Nevertheless, the host plant location behaviour of *M. aeneus* depends on several characteristics of host plants including colour, odour, chemical compounds, physical architecture etc. (Blight and Smart 1999; Charpentier 1985; Cook et al. 2007a,b, 2013; Ekbom and Borg 1996; Jönsson 2005). The quality of the host plant is considerably influenced by defensive mechanisms in the plant such as secondary compounds, and by the nutritional value of the plant tissues. Selection and acceptance of plants for oviposition by most herbivorous insects are based on chemical stimuli from the plants (Renwick and Chew 1994). Pollen beetles are attracted to metabolites of glucosinolates, such as isothicyanates and nitriles, as well as to non-specific volatiles that are released from plants of various plant families (Cook et al. 2007a). The presence of different glucosinolate profiles in different host plants might explain why the female shows preferences for particular plant species (Ekbom 1998); the secondary compounds of host plants may deter insect feeding or cause greater larval mortality.

Clutch size

One of the characteristics showing pollen beetles' host plant preferences is clutch size as pollen beetles may reduce egg number per clutch on low quality host plants (Hopkins and Ekbom, 1996). In our study, the mean number of eggs found in green buds, was greatest on *B. nigra*, which differed significantly from all other plant species. However, there were some differences between years. In 2011, *B. nigra* was highly preferred over the other plant species and also there were more eggs in other studied plants from the genus Brassica with only few eggs found in buds of *S. alba* and no single eggs from *E. sativa*. In 2012, surprisingly, the most preferred plant species was *R. sativus*, which differed significantly from other studied plant species except *B. napus*. We can assume that because of weather conditions of 2012 (constant low temperatures during April and May did not favour the emergence of *M. aeneus* from its hibernation sites) the phenology of *M. aeneus* appeared better synchronised with the phenology of *R. sativus* than that of *B. nigra*, *B. rapa* or *B. juncea*. In previous studies, there have been significantly fewer larvae per plant on *R. sativus* than on *B. nigra* (Veromann et al. 2012) and no differences between *B. napus* and *R. sativus* (Veromann et al. 2012; Kaasik et al. 2014). In addition, contrary to our findings, Ekbom (1998) found in laboratory experiments that plants belonging to the Brassica genus are more attractive to the beetles than crucifers from other genera. On the other hand, field experiments have shown the potential of *R. sativus* as a trap crop to lure cabbage flea beetle away from cabbage and similarly to our study, *R. sativus* was preferred over *B. nigra* and *B. napus* (Bohinc and Trdan 2013). Therefore, this plant species can attract beetles under certain conditions. Although pollen beetles are highly attracted to the colour yellow, they preferred to lay eggs on *R. sativus* which has violet flowers. However, *R. sativus* buds are green and/or yellowish, so the colour of buds did not play a crucial role in oviposition behaviour of pollen beetles in our study. The unattractiveness of *E. sativa* and *S. alba* for egg laying of pollen beetles have been shown by several studies before (Ekbom 1998; Ekbom and Borg 1996; Veromann et al. 2012; Kaasik et al. 2014). These plants may be therefore used primarily as a food source

by pollen beetles (Ekbom and Borg 1996). The attractiveness of *B. nigra* over *B. napus* and *B. rapa* contrasts with the results of Borg and Ekbom (1996), Ekbom and Borg (1996) and Ulber and Thieme (2007) who found that *B. nigra* was less attractive than *B. napus* for oviposition by pollen beetles. However, our results confirm the findings of Veromann et al. (2012) who reported significantly more larvae on *B. nigra* and Kaasik et al. (2014) who found no differences in attractiveness between these plant species.

The data on the number of eggs per bud was similar at yellow bud and green bud stages and greatest in *B. nigra* in 2011 but in *B. juncea* in 2012. The number of larvae found on *R. sativus* was similar to that on *B. napus*, *B. rapa*, *B. juncea* and *B. nigra*. Therefore, *R. sativus* was as attractive a Brassica plant species for oviposition by pollen beetles but, as almost half of the larvae died, it acted as a dead-end trap crop.

In conclusion, besides being equally attractive for oviposition by *M. aeneus* as *B. napus* during the most damage susceptible stage of plants – the green bud stage –, *R. sativus* also performed as a potential dead-end trap-crop because almost half the larvae failed to survive. This creates an interesting situation for insect pest management: planting *R. sativus* near to the fields with commercial cruciferous plants would not only protect the crops against *M. aeneus*, but may also reduce populations of this pest by killing the larvae. However, *R. sativus* must be evaluated for its effects on other insects, diseases and weed management before it can be recommended in an overall pest management program. Secondly, laboratory experiments are needed to study larval survivability on different host plants. Also additional studies are needed to determine the survival rate of pupae and fecundity of *M. aeneus* developed on alternative host plants. Furthermore, *B. nigra* and *B. rapa* were significantly preferred to *B. napus* and therefore the potential for using these plant species as trap crops for spring rape need to be tested in large field trials and under local conditions.

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Effects of nitrogen fertilization on insect pests, their parasitoids, plant diseases and volatile organic compounds in *Brassica napus*

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ABSTRACT

Nitrogen (N) availability is a key factor influencing the yield of *Brassica napus* L. Thus, mineral fertilization is widely used to improve the quality and quantity of seeds. In this study, we conducted field experiments to determine the impact of nitrogen fertilization on *B. napus* pests, their parasitoids and plant diseases. The results showed that N treatment had an impact on the abundance of pollen beetles (*Meligethes aeneus* Fab.) and cabbage seed weevils (*Ceutorhynchus obstrictus* Marsh.) as well as dark spot disease (*Alternaria brassicae* (Berk.) Sacc.). Since pest abundance was not correlated with the flower and silique numbers, the feeding and oviposition sites, plant smell bouquets were analysed to determine potentially attractive or repellent volatile organic compounds. We detected 19 different compounds among which acetic acid and several lipoxygenase pathway products were emitted at higher levels from N-treated plants. Emission of a few other terpenoid compounds was correlated with the pest abundance in field conditions. Abundance of parasitoids of both pests was related to the host availability rather than to the fertilization treatment. Therefore, we suggest that plant chemical cues play a minor role in localization of hosts in close proximity to parasitoid. Dark spot disease levels decreased with increasing N availability, possibly reflecting enhanced emissions of acetic acid, a known antifungal volatile. This study demonstrates the effects of N fertilization on bud and flower volatile bouquets, which might play a role in *B. napus* insect pest host selection and in resistance to fungal plant diseases. Further studies are necessary to investigate the behavioural responses of insects to the changed volatile bouquets.

1. Introduction

Oilseed rape (*Brassica napus* L.) is a widely cultivated field crop in temperate climates (Blake et al., 2010) where its growth and development is most commonly limited by nitrogen (N) availability, especially at the outset of vegetative growth (e.g. Holmes, 1980; Rathke et al., 2006; Sieling and Kage, 2010). Conventionally, mineral fertilizers are applied to improve the yield and biochemical characteristics of this important agricultural crop. Fertilization with mineral N has been shown to increase plant size, height and inflorescence branching as well as seed protein content (Allen and Morgan, 1972; Blake et al., 2010; Grant et al., 2010; Holmes, 1980). However, due to economical and ecological reasons, the fertilization should be conducted carefully to apply only the amount necessary for optimal plant growth. Several studies have demonstrated that excessive N application, especially in *B. napus* cropping systems, often leads to N-leaching which causes soil and water pollution (Engström et al., 2011; Sieling and Kage, 2010). Application of only optimal fertilizer doses is also important for yield quality, since increasing N levels can result in decreased seed oil content (Grant et al., 2010). Although numerous research groups have focused on finding optimal N doses for improved yields and seed quality (e.g. Colnenne et al., 1998), very little is known about the impact of fertilization on *B. napus* insect pests and diseases or on other organisms associated with this cropping system. However, the interactions of *B. napus* with other organisms may considerably affect the yield as well as the quality of this crop throughout the growing season.

Among *B. napus* pests, the pollen beetle (*Meligethes aeneus* Fab., Coleoptera: Nitidulidae) and the cabbage seed weevil (*Ceutorhynchus obstrictus* Marsh., syn. *Ceutorhynchus assimilis* Payk.), (Coleoptera: Curculionidae) are the most widespread and persistent pests in the UK, Central, North and North Eastern Europe (Alford et al., 2003; Veromann et al., 2006b; Williams, 2010).

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Pollen beetle adults feed on pollen, destroy flower buds and lay eggs into the buds where their larvae feed on pollen. Seed weevils oviposit in developing siliques and their larvae consume seeds. The management of these *B. napus* pests is generally conducted by chemical pesticides, although their abundance could be efficiently controlled by their natural enemies, parasitoids (Büchi, 2002; Veromann et al., 2011; Walters et al., 2003). Parasitoids are insects, mostly from the order Hymenoptera, laying eggs on or inside the insects of other species. Their larvae develop and feed on the host, eventually killing it (Godfray, 1994). In Europe, the larvae of *M. aeneus* are parasitized by at least nine species of endoparasitoids. Among these, *Phradis interstitialis* (Thomson), *Phradis morionellus* (Holmgren), *Tersilochus heterocerus* (Thomson) and *Diospilus capito* (Nees) (Hymenoptera, Ichneumonoidea) are the most abundant and widespread (Nilsson, 2003). The most common parasitoids of *C. obstrictus* are larval ectoparasitoids belonging to the superfamily Chalcidoidea: *Trichomalus perfectus* (Walker), *Mesopolobus morys* (Walker) and *Stenomalina gracilis* (Walker) (Williams, 2003).

Among *B. napus* diseases, the most devastating are blackleg (*Leptosphaeria maculans* (Desm.) Ces.) and stem rot (*Sclerotinia sclerotiorum* (Lib.) de Bary). Additionally, the dark spot disease (*Alternaria brassicae* (Berk.) Sacc.) is a common fungal pathogen, especially on winter varieties (Giamoustaris and Mithen, 1997; Köhl et al., 2010). Disease control in conventional cropping systems is mainly conducted by preventive fungicide applications throughout the season, often without considering the presence or abundance of pathogens.

So far, it has been assumed that N usage is unlikely to have any direct effect on the abundance of the *B. napus* insect pests (Walters et al., 2003) and only a limited number of studies has focussed on the effects of N fertilization on parasitism level of some lepidopterans' and aphids' pests (Jansson, 2003; Kalule and Wright, 2002; Stadley et al., 2011). For instance, the amount of nitrogen supplied to *Brassica oleracea* var. *capitata* has been shown to increase the parasitism level of *Plutella xylostella* L. (Lepidoptera: Plutellidae) by *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae) (Jansson et al., 1991). However, fertilization changes plant architecture and inner microclimate of the crop, which might influence the searching efficiency of both the insect pests and their hymenopterous parasitoids (Walters et al., 2003). Additionally, plant architecture can have an impact on the distribution and accessibility of host larvae within the stand and therefore may affect the host location success of the parasitoids (Ulber and Fisher, 2006). There is some evidence of either negative or positive effects of N on stem rot disease and blackleg (Aubertot et al., 2003; Rathke et al., 2006; Söchting and Verreet, 2004), although the impact of fertilization on dark spot disease was non-significant in previous experiments (Söchting and Verreet, 2004).

Chemical communication between herbivores and their host plants depends on the plant and herbivore species and is generally based on multiple compounds (Blight et al., 1997). Cruciferous plants, such as *B. napus*, emit a complex mixture of biogenic volatile organic compounds (VOCs). Among them, isothiocyanates from foliage and floral compounds are known to be important cues in host selection by cruciferous pests and their parasitoids, aiding both finding and recognition of the host plant (Alford et al., 2003; Bartlet et al., 1993; Schiestl, 2010). Nitrogen fertilization could significantly affect the composition and levels of plant VOCs (Chen et al., 2010) and therefore also affect their attractiveness to pests. Our preliminary results on the effect of N show that there might be a correlation between the nutrient application and the abundance of pests, diseases and even parasitoids (Veromann et al., in press). Thus, it is necessary to conduct additional experiments to determine the factors involved in the pest and disease abundance, which could be further used to control the damage to the crop without the use of pesticides.

In this study we hypothesized that N fertilization affects the abundance of pests and parasitoids, as well as the spread of plant diseases, and that these potential modifications are associated with changes in the VOC bouquets of the plants. To test this hypothesis, we determined the VOC emissions of *B. napus* plants under laboratory conditions and compared these with the field data on abundance of insect pests, their parasitoids and plant diseases under different N treatments. The results of this study provide evidence that the composition of the VOCs emitted from *B. napus* plants is linked to the N fertilization effects on insect damage and dark spot disease.

2. Material and methods

2.1. Field experiment setup and yield estimation

The field studies were conducted in an experimental field of Jõgeva Plant Breeding Institute, Estonia (58°46′01″N, 26°24′27″E; elevation 74 m) in 2008 and 2009. The study site was ploughed and kept as a fallow for one growing season before starting this experiment. On the 23 April of each year, seven different N-fertilizer levels of 0, 60, 80, 100, 120, 140 and 160 kg of N (applied as NH_4NO_3 (Akron Group, Novgorod, Russia)) per hectare were applied on three randomized replicate plots of 10 m^2. Thus, the study design consisted of seven nitrogen treatments (hereinafter indicated as N0, N60, N80, N100, N120, N140, N160) for a total of 21 plots. Before sowing the seed in 2007 and in 2008 (for the 2008 and 2009 harvest, respectively), all the plots were fertilized with Kemira Power (Yara International ASA, Norway) 5–10–25 S Fe B complex fertilizer (300 kg ha^{-1}). In both years, a winter oilseed rape variety *B. napus* cv. *Silva* seeds were drilled on the 15 August in 2007 (for 2008 harvest) and on the 15 August in 2008 (for 2009 harvest) with seeding rate of 6 kg ha^{-1}. No insecticides or fungicides were applied.

The crop was harvested on the 7 September in 2008 and on the 11 August in 2009. The seed mass was measured for every plot by weighing the seeds at a moisture content of 7.5%. Thereafter, the yield per each plot was calculated as kg ha^{-1}. In addition, the number of siliques was counted. Silique counting was conducted at the pods ripening stage (plant growth stage BBCH 80–81 according to Lancashire et al., 1991) on three plants from each plot.

2.2. Quantification of pests, parasitoids and plant diseases

For estimation of egg laying activity and larval parasitization levels of *M. aeneus*, larvae were collected from *B. napus* flowers at stage BBCH 67–68 (at the end of full flowering) from five randomly chosen plants in each plot. Larvae were counted and second instar larvae were dissected in laboratory to determine their parasitization. Second instar is the last *M. aeneus* larval stage before dropping to the ground to pupate in the soil. Thus, by this time their parasitoids must have had found a suitable host to parasitize.

The establishment of damage and parasitization of *C. obstrictus* was assessed at stage BBCH 80–81 (beginning of pods ripening). Five siliques from the main raceme and five siliques from the third side branch were collected from five randomly chosen plants per plot and incubated in emergence traps in the laboratory as described in detail in Veromann et al. (2011). Four weeks later, emerged adult parasitoids or weevils' larvae were counted and identified. The exit-holes of larvae and parasitoids in all siliques were counted. Thereafter, all siliques were dissected and remains of any non-exited weevil larvae or the parasitoid pupae were noted. Finally, the mean number of emerged parasitoids, damaged siliques

by *C. obstrictus* and the percentage of parasitized weevils were calculated per plant.

Visual disease assessments were made at the mature pod stage (BBCH 80–85) when five plants were randomly chosen from every plot. The abundance of *A. brassicae* lesions on leaves, stems and siliques was visually assessed on a qualitative scale from 0 to 6 (0 – no disease; 1 – 1–5%; 2 – 5–10%; 3 – 10–20%; 4 – 20–30%; 5 – 30–50%; 6 – more than 50% of surface area covered with lesions), based on the assessment key illustrated in Conn et al. (1990).

2.3. Volatile organic compound measurements

The VOC measurements were carried out only in May 2010 using the plants from the same *B. napus* variety grown in similar experimental setup as described above. Since the plants were treated similarly to the ones in field studies in 2008 and 2009, we used the data from VOC analyses to determine potential volatile cues in plant–insect relationships and disease abundance that were detected in field studies during earlier years. To detect and compare VOCs from plants with different fertilization levels, five well overwintered plants from treatments N0, N80, N100 and N160 were taken from the field on the 4th May (stem elongation stage BBCH 33–35). The plants were planted in 5 L pots with the soil from the field and placed in a growth room where they were protected from biogenic stressors and watered every second day. The plants were grown under metal halide lamps (HPI-T Plus 400 W, Philips). At plant level, the light intensity was 300 μmol m^{-2} s^{-1} for 12 h light period and day/night temperatures of 24/18 °C.

The first set of VOC measurements was made on the 17th May, when the plants were in bud stage (BBCH 51–53) and the second set of measurements on the 20th May when the plants were flowering (BBCH 63–65). For both sets of measurements, three plants from every treatment were chosen with similar number of buds or flowers. The measurements were carried out in the morning, between 9.00 a.m. and 11.00 a.m. The top of main raceme of one plant from every treatment at a time was placed into a multi-chamber cuvette system described in Toome et al. (2010) and the volatiles were sampled by concentrating the air from every chamber into a multibed stainless steel cartridge. The adsorption was carried out at a flow rate of 200 ml min^{-1} for 20 min with a constant flow air sample pump (SKC Inc., Houston, TX, USA). Thereafter the same procedure was repeated with the other two sets of plants, each containing one plant from every N0, N80, N100 and N160 treatment. Additional samples were taken from the inlet air stream prior to the chambers to determine the background value of the VOCs.

The adsorbent cartridges were analysed with a combined Shimadzu TD20 automated cartridge desorber and Shimadzu 2010 plus GC MS instrument (Shimadzu Corporation, Kyoto, Japan) using the method described in detail previously (Copolovici et al., 2009, 2011; Toome et al., 2010). The compounds were identified by comparing their mass spectra with a NIST library (National Institute of Standards and Technology) and with authentic standards. VOC emission rates were expressed per bud or flower projected area enclosed in the chamber.

2.4. Data analysis

The differences among the means of the number of siliques, yields, damaged siliques, parasitoids of *C. obstrictus* and larvae of *M. aeneus* between the treatments were tested by Wald Statistics using Type III empirical standard error analysis with the Poisson distribution and the *log*-link function. Differences among the means between years and treatments were tested by the GENMOD procedure Differences of Least Squares Means test. The average numbers of parasitized larvae of *M. aeneus* were compared by the same analysis, but with Binomial distribution and *logit*-link function with second instar larvae as the response variable. The scale parameter was estimated by Pearson Chi-Square divided by the degrees of freedom to account for the model overdispersion. Spearman correlation coefficient r was calculated to test for the correlations between the number of *M. aeneus* larvae and their parasitism level, and between damaged siliques and parasitism level of *C. obstrictus* larvae. Additionally, correlations between the abundance of both pests and the volatile emission rates measured in the laboratory conditions were calculated. The impact of treatment on the mean disease scores was determined with a nonparametric Kruskal–Wallis Test. In addition, Spearman correlation coefficients were calculated to describe the correlation between the fertilizer application level and disease scores. These statistical analyses were carried out using GLM and GENMOD procedures in SAS 8.02 (SAS Institute, Inc., Cary, NC, USA).

The impact of nitrogen fertilization on the emission of various volatiles was visualized by fitting data in a non-linear regression model in the exponential form, using OriginPro 8 (OriginLab Corporation, Northampton, MA, USA). The smell bouquets of volatiles (based on the emission rates) released by main racemes at bud and flowering stage were explored by principal component analysis (PCA) (Wold et al., 1987). After mean-centering, square-root data transformation was used. The resulting loading and score plots were derived for volatile bouquets at bud and flower stages for different N treatments. The difference between the emission bouquets from plants at flowering and bud stage was tested by Monte-Carlo permutation test using redundancy data analysis (RDA). The multivariate analyses were conducted by Canoco 4.5 software (ter Braak and Smilauer, Biometris Plant Research International, The Netherlands). All statistical tests were considered significant at $P < 0.05$.

3. Results

3.1. Impact of fertilization on the number of siliques

Fertilization significantly increased the number of siliques and yield (Table 1). Plants in control plots with no external N-supply had the smallest number of siliques and the smallest yield ($\chi^2 = 53.49$, $df = 6$, $P < 0.0001$). The highest number of siliques was found from treatments N100 to N160 with no significant differences between these treatments (Table 1). The plants in N160 plots had nearly three times more siliques than the plants growing in N0 plots ($\chi^2 = 46.76$, $df = 1$, $P < 0.0001$). Overall, the ranking of treatments according to the number of siliques and yield corresponded to the fertilization level, although not always. Year effect was significant in most cases for yield and for N120 for number of siliques, but the qualitative trend of increased number of siliques and yield was the same in both years (Table 1).

3.2. Insect pests and their parasitoids

Overall, the oviposition activity of *M. aeneus* as well as the parasitism level of their larvae was relatively low. The mean number of *M. aeneus* larvae ($\chi^2 = 63.80$, $df = 6$, $P < 0.0001$) and parasitized larvae per plant ($\chi^2 = 78.45$, $df = 6$, $P < 0.0001$) differed significantly among N fertilization treatments (Fig. 1a, b, c). Although the number of larvae was significantly greater in 2009 compared to 2008 ($\chi^2 = 32.18$, $df = 1$, $P < 0.0001$), the general distribution of larvae between treatments had a similar pattern (Fig. 1a, b). In 2008, the number of larvae per plant was extremely low and only the treatments N80 ($\chi^2 = 3.75$, $df = 1$, $P = 0.05$) and N160 ($\chi^2 = 4.54$, $df = 1$, $P = 0.03$) had significantly greater number

Table 1
Mean (±SE) number of siliques per plant and average yield under different N-fertilization treatments in winter oilseed rape (*Brassica napus* cv. *Silva*) in two growing seasons.

Treatment kg N ha^{-1}	Number of siliques per plant			Yield (kg ha^{-1})		
	Year		Mean	Year		Mean
	2008	2009		2008	2009	
0	100 ± 10 Aa	75 ± 13 Aa	87 ± 8 a	1370 ± 240 Aa	1670 ± 30 Aa	1520 ± 130 a
60	153 ± 18 Aabc	204 ± 27 Abc	178 ± 17 b	2350 ± 570 Ab	4910 ± 80 Bb	3630 ± 630 b
80	188 ± 35 Abcd	192 ± 17 Abc	190 ± 19 bc	2940 ± 620 Abc	4920 ± 170 Bb	3930 ± 530 bc
100	217 ± 37 Abcde	244 ± 36 Ac	230 ± 25 cd	3290 ± 530 Abc	4900 ± 120 Bb	4090 ± 430 bc
120	247 ± 35 Ade	172 ± 15 Bb	204 ± 23 bcd	3320 ± 480 Ac	5230 ± 300 Bb	4280 ± 500 bc
140	250 ± 19 Ade	206 ± 21 Abc	228 ± 15 bcd	3030 ± 130 Abc	4810 ± 110 Bb	3920 ± 400 bc
160	286 ± 26 Ae	230 ± 30 Abc	258 ± 20 d	3640 ± 270 Ac	5200 ± 240 Bb	4420 ± 380 c

Capital letters indicate significant differences between years within given treatment and lowercase letters among the treatments within the given year ($P < 0.05$ according to GENMOD Differences of Least Squares Means test).

of larvae per plant than N0 (Fig. 1a). In 2009, the number of larvae was greater and the treatments N60, N80 and N160 clearly differed from the remaining treatments ($P < 0.01$; Fig. 1b). Similarly to the larvae, the parasitism percentage differed significantly between the years ($\chi^2 = 38.32$, $df = 1$, $P < 0.0001$; Fig. 1a, b). In 2008, the maximum percentage reached to 9.76 in N160 and differed significantly from N80 ($\chi^2 = 4.79$, $df = 1$, $P < 0.05$; Fig. 1a), whereas in 2009, parasitism percentage reached 47.62 in N0, differing significantly from all other treatments, except for the treatment N80 ($\chi^2 = 2.74$, $df = 1$, $P > 0.05$; Fig. 1b). When analysing the data from two years together, the greatest numbers of *M. aeneus* were found in plots N60, N80 and N160 ($P < 0.05$; Fig. 1c). Although the smallest number of larvae was found in the flowers of the plants from treatment N0, no significant difference between larval number was detected between the treatments N0 and N140 ($\chi^2 = 2.00$, $df = 1$, $P > 0.05$). The mean parasitism rate averaged across years studied was the greatest in the least *M. aeneus* infested plants in plots N0, where 26% of larvae were parasitized. A statistically significant difference in parasitism level was found only between treatments N0 and N100 ($\chi^2 = 4.71$, $df = 1$, $P < 0.05$; Fig. 1c). A significant positive correlation was detected between the number of pollen beetle larvae and the number of parasitized larvae ($r = 0.24$, $P < 0.01$).

The mean percentage of siliques damaged by *C. obstrictus* varied from 8.3 to 14.3%. Fertilization with nitrogen affected significantly the number of damaged siliques per plant averaged across years ($\chi^2 = 12.71$, $df = 6$, $P < 0.05$; Fig. 1f). Averaged across years, the mean number of *C. obstrictus* damaged siliques was higher in the treatment N80 than in the treatments N100 ($\chi^2 = 7.64$, $df = 1$, $P < 0.01$) and N140 ($\chi^2 = 7.64$, $df = 1$, $P < 0.01$; Fig. 1f). In 2008, the number of siliques damaged by *C. obstrictus* was extremely low and due to high variability there were no significant differences between the treatments (Fig. 1d). In 2009, significantly more damaged siliques was found than in 2008 ($\chi^2 = 9.51$, $df = 1$, $P < 0.01$; Fig. 1e). The least damaged plants were in plots N100 and N140 and they differed significantly from the treatment N80 (accordingly: $\chi^2 = 6.34$, $df = 1$, $P < 0.05$ and $\chi^2 = 3.80$, $df = 1$, $P = 0.05$). The mean parasitism percentage of the *C. obstrictus* larvae was extremely high, varying from 76% to 94% with an average of 86.4 ± 2.8%. In 2008–2009, the greatest number of parasitoids was found in the treatments N60 ($\chi^2 = 4.31$, $df = 1$, $P < 0.05$), N80 ($\chi^2 = 5.54$, $df = 1$, $P < 0.05$) and N120 ($\chi^2 = 3.93$, $df = 1$, $P < 0.05$), where significantly more parasitoids emerged from siliques than in plot N140 (Fig. 1f). A strong positive correlation between host availability (damaged siliques) and parasitism level was found across all nitrogen treatments when pooled ($r = 0.94$, $P < 0.0001$). In 2008, the number of emerged parasitoids was equal with the number of damaged siliques almost in all treatments. Thus, the parasitism level was 100% and there were no differences between treatments (Fig. 1d). In the next study year, the number of emerged parasitoids was somewhat smaller and the parasitism level varied between 63.9 and 95.5% (Fig. 1e). There were no differences between the two study years ($\chi^2 = 2.21$, $df = 1$, $P = 0.14$).

3.3. Diseases

The only *B. napus* disease found during this two-year study was dark spot disease, a fungal infection caused by *A. brassicae*. This disease occurred on leaves, stems and siliques. The average disease score was rather low (Fig. 1h), yet it significantly depended on nitrogen fertilization ($\chi^2 = 64.90$, $df = 6$, $P < 0.0001$). During the first study year, the highest disease scores were detected in plots N0 and N60 and the plants treated with greater nitrogen levels had significantly less disease lesions ($P < 0.05$; Fig. 1g). In the following year, there was no difference between the control plants and plants with highest nitrogen treatment. However, plants in N80 plots were the least infected (compared to N0: $\chi^2 = 64.90$, $df = 1$, $P < 0.01$; N60: $\chi^2 = 4.48$, $df = 1$, $P < 0.05$; N100: $\chi^2 = 4.48$, $df = 1$, $P < 0.05$; N120: $\chi^2 = 6.29$, $df = 1$, $P < 0.05$; N140: $\chi^2 = 13.12$, $df = 1$, $P < 0.01$; N160: $\chi^2 = 28.17$, $df = 1$, $P < 0.01$).

3.4. Emissions of volatile organic compounds in relation to N treatment

Altogether 19 different VOCs were detected from the oilseed rape racemes during this study (Table 2). The highest emission rates were detected for acetic acid, 3-carene, α-pinene and benzaldehyde (Table 2). These compounds dominated the emissions from plants in both bud and flower stages. For individual compounds, the treatment effect was moderate or small in most cases (Table 2). Nevertheless, emissions of several compounds increased significantly with increasing N availability, in particular LOX, α-thujene and acetic acid emission rates (Fig. 2).

The bouquets were not clearly separated among different N treatments as the bouquets from N-treated plants clustered together with controls on PCA (Fig. 3). Plants in flowering stage treated with higher N levels clustered separately from controls, reflecting their greater (3*Z*)-hexenol, α-thujene and acetic acid emission rates (Fig. 3).

In average, several statistically significant correlations between the bud stage volatiles and abundance of larvae of *M. aeneus* and between the flower stage VOC and the damaged siliques by *C. obstrictus* were found (Table 3). In particular, there were significant negative correlations between the abundance of *M. aeneus* larvae and β-pinene, linalool, (E,E)- α-farnesene, benzaldehyde and methylbenzoate. Larval abundance was positively correlated with (3*Z*)-hexenyl acetate, camphene, 3-carene, limonene, indole and acetic acid emission rates. Statistically significant but weak positive

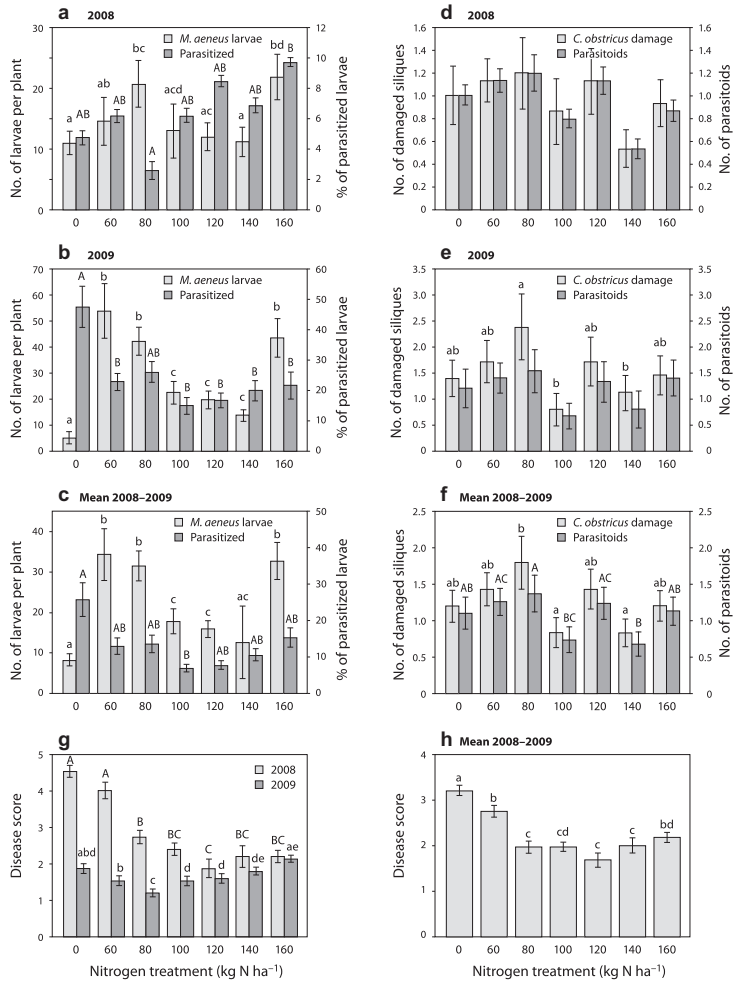

Fig. 1. *Brassica napus* pests, their parasitoids and diseases at seven different N fertilization levels in the field experiment in Jõgeva County, Estonia, 2008–2009. – a, b, c – Mean number of *Meligethes aeneus* larvae per plant (light grey) and the percentage of parasitized larvae (dark grey) in 2008, 2009, and as a mean of two study years; d, e, f – Mean number of siliques per plant damaged by *Ceutorhynchus obstrictus* (light grey) and the number of parasitoids emerged from siliques (dark grey) in 2008, 2009, and as a mean of two study years; g, h – Mean disease score for *Alternaria brassicae* in 2008 and 2009, and as a mean of two study years. Letters show significant differences between treatments within the data series at $P < 0.05$. The error bars represent ± SE.

correlations were found between α-pinene, camphene, β-pinene emissions and the abundance of damaged siliques by *C. obstrictus*. There were some differences among years 2008 and 2009 in the correlations of the bud stage volatiles vs. the abundance of larvae of *M. aeneus*. In the first year, significant correlations of larval abundance vs. β-pinene, linalool, (E,E)- α-farnesene, benzaldehyde and methylbenzoate were observed. In 2009, α-pinene not correlated with larval abundance (Table 3). In that year, statistically significant

Table 2
Average (±SE) emission rates (pmol m^{-2} s^{-1}) of key volatile organics from *Brassica napus* cv. *Silva* plants at floral bud and flower stages in control, medium and high N treatments measured under control conditions. The averages are calculated based on all four treatments measured (N0, N80, N100 and N160).

Compound	Floral bud stage					Flower stage				
	Average ± SE	N treatment (kg N ha^{-1})				Average ± SE	N treatment (kg N ha^{-1})			
		0	80	100	160		0	80	100	160
Green leaf volatiles (lipoxygenase pathway volatiles, LOX)										
(3*Z*)-hexenol	5.0 ± 1.4A[a]	3.8 ± 1.4a	4.5 ± 0.5a	8.8 ± 4.4a	4.0 ± 2.4a	9.8 ± 2.7A	3.1 ± 0.8a	13 ± 6b	6.5 ± 2.1a	17 ± 7b
(2*E*)-hexenal	4.1 ± 1.0A	3.31 ± 0.38a	2.6 ± 0.8a	6.3 ± 2.2a	4.1 ± 2.0a	2.22 ± 0.38B	1.00 ± 0.04a	3.5 ± 0.5b	2.1 ± 0.8b	2.3 ± 0.5b
(3*Z*)-hexenyl acetate	38 ± 12A	15.4 ± 2.1a	28 ± 7a	41 ± 27a	69 ± 22b	17.1 ± 2.6B	9.1 ± 1.4a	25 ± 6b	12.6 ± 2.5a	22.2 ± 3.1b
1-hexanol	16.1 ± 3.6A	10.4 ± 0.7a	9.8 ± 0.9a	22 ± 12a	19 ± 6b	7.1 ± 1.7B	4.7 ± 1.5a	6.8 ± 4.5a	5.7 ± 2.8a	11 ± 3a
Monoterpenes and derivatives										
α-thujene	nd[b]	nd	nd	nd	nd	0.90 ± 0.30	0.31 ± 0.14a	0.9 ± 0.6b	1.0 ± 0.4b	1.3 ± 0.5b
α-pinene	80 ± 21A	70 ± 25 ab	75 ± 18 ab	126 ± 49a	51 ± 29b	34 ± 5B	29.8 ± 1.6a	45 ± 15b	21 ± 9a	39 ± 11b
Camphene	17 ± 12A	4.3 ± 1.1a	5.9 ± 0.8a	7.4 ± 1.5a	51 ± 27b	2.49 ± 0.39B	2.5 ± 0.7a	3.2 ± 1.3a	1.9 ± 0.6a	2.32 ± 0.34a
β-pinene	22 ± 6A	19 ± 8a	17.4 ± 4.6a	35 ± 15a	17 ± 9a	8.8 ± 1.4B	7.5 ± 1.2a	11.6 ± 3.3a	4.3 ± 1.2a	11.6 ± 2.5a
3-carene	108 ± 19A	86 ± 18a	107 ± 10a	143 ± 45a	98 ± 23a	57 ± 13B	35 ± 7a	73 ± 17b	33 ± 14a	85 ± 18b
Limonene	20.2 ± 2.9A	15.0 ± 2.0a	24 ± 7a	24 ± 8a	17.9 ± 4.3a	27 ± 11A	9.1 ± 2.3a	35 ± 27b	15 ± 4b	50 ± 34b
Linalool	1.05 ± 0.30A	1.6 ± 0.7a	0.60 ± 0.02a	1.6 ± 0.9a	0.47 ± 0.47a	1.32 ± 0.24A	0.83 ± 0.12a	1.03 ± 0.36a	1.8 ± 0.7a	1.64 ± 0.31a
Geranyl acetone	18.0 ± 4.9A	16.9 ± 3.4a	12.7 ± 2.9a	24 ± 12a	18 ± 15a	8.6 ± 2.4A	8.4 ± 1.5a	16 ± 7a	6.0 ± 2.4a	3.4 ± 1.3a
Sesquiterpenes										
(*E*,*E*)-α-farnesene	1.11 ± 0.21A	1.04 ± 0.42a	0.99 ± 0.12a	1.6 ± 0.5a	0.8 ± 0.5a	0.51 ± 0.13A	0.67 ± 0.18a	0.36 ± 0.18b	0.85 ± 0.30a	0.15 ± 0.15b
Aromatics										
Benzaldehyde	82 ± 10A	89 ± 32a	66 ± 9a	89 ± 20a	76 ± 18a	63 ± 15A	49 ± 10a	62 ± 32a	54 ± 26a	85 ± 50a
Benzyl alcohol	1.72 ± 0.40A	1.46 ± 0.42a	0.89 ± 0.16a	2.5 ± 1.3a	2.0 ± 0.8a	1.26 ± 0.13A	1.23 ± 0.17a	1.30 ± 0.24a	1.08 ± 0.43a	1.43 ± 0.19a
Indole	0.17 ± 0.10A	nd	nd	0.33 ± 0.21	0.37 ± 0.23	0.38 ± 0.16A	0.13 ± 0.07a	0.29 ± 0.22a	0.9 ± 0.5a	0.15 ± 0.15a
Methylbenzoate	0.88 ± 0.29A	1.6 ± 0.8a	0.09 ± 0.07b	1.56 ± 0.22a	0.29 ± 0.14b	2.11 ± 0.47A	2.2 ± 0.5a	2.1 ± 0.7a	1.7 ± 0.8a	2.5 ± 1.9a
Methyl salicylate	1.21 ± 0.23A	1.01 ± 0.14a	0.56 ± 0.23a	1.78 ± 0.46b	1.50 ± 0.46b	0.36 ± 0.08B	0.22 ± 0.10a	0.23 ± 0.09a	0.41 ± 0.14b	0.58 ± 0.14b
Carboxylic acids										
Acetic acid	350 ± 120A	78 ± 16a	310 ± 60 ab	490 ± 150b	500 ± 160b	750 ± 180B	400 ± 90a	700 ± 270b	660 ± 170b	1250 ± 250c

Capital letters denote significant differences between the means of bud and flower stages, while lowercase letters denote significant treatment differences within the growth stage.
[a] Means were compared by ANOVA followed by LSD tests.
[b] nd – below the detection limit.

but weak positive correlations were found between the abundance of damaged siliques by *C. obstrictus* and the emission of α-pinene, camphene and β-pinene.

3.5. Differences in emission signatures between bud and flower stages

Important differences in VOC emissions from racemes were detected between the bud and flower stages (Table 2 for full statistical analysis). For instance, α-thujene emission was detected only while the plants were flowering and not in the bud stage. Additionally, the mean emissions of several other monoterpenes, i.e., α-pinene ($F_{(2,2)} = 17.64$, $P < 0.05$), β-pinene ($F_{(2,2)} = 18.37$, $P < 0.05$), 3-carene ($F_{(2,2)} = 2.14$, $P < 0.05$), were detected at considerably higher rates during the bud stage compared to the flower stage. The mean emission rate of acetic acid was higher in the flowering stage than in the bud stage. In contrast, the emission rates of camphene and methyl salicylate were higher in the bud stage compared to the flowering stage ($F_{(2,2)} = 946.70$, $P < 0.05$ and $F_{(2,2)} = 8.26$, $P < 0.05$, respectively; Table 2).

PCA analysis demonstrated that VOC bouquets of *B. napus* plants at bud and flowering stage are clearly separated (Fig. 3) and statistically different (Monte Carlo permutation test, $P < 0.05$). There was a large variation within the bouquets of volatiles released at these two different stages. The distribution of plants on score plot was mainly influenced by the emission rate of (3*Z*)-hexenyl acetate, α-pinene, camphene, 3-carene, benzaldehyde and acetic acid (Fig. 3).

4. Discussion

It is known that many insect pests select their hosts based on visual and semiochemical stimuli (Cook et al., 2007a,b). Our field study results show that N fertilization affected pest host selection, although not in a simple linear manner. For both studied herbivorous insects, plants at moderate N treatments (N100–N140) were less attractive than plants treated with low or high N levels. Since the number of flowers and therefore the availability of suitable oviposition and feeding sites increased monotonically with added nitrogen, it could be suggested that host selection was possibly more affected by semiochemical stimulants than the flower abundance. However, additional laboratory experiments are needed to provide insight into the insect behavioural responses to altered volatile bouquets.

For *M. aeneus* females, the most preferred stage for oviposition is the bud stage with buds of only 2–3 mm in size (Borg, 1996; Nilsson, 1988). Therefore, the chemical components emitted by buds should be an important cue for the insects in locating *B. napus* plants. Measurements of VOCs in controlled environments detected a significant positive effect of N on acetic acid emission in the bud stage. Acetic acid has been demonstrated as product and intermediate in the biosynthesis of secondary metabolites in *Brassica rapa* (Abdel-Farid et al., 2007). Since the control plants were also less infested with the beetle larvae, acetic acid may be one of the cues for the females. Methyl salicylate emission was also detected at increased levels in plants treated with high N levels (N160) at both bud and flowering stages. This compound has been shown in numerous experiments to be attractants for sap-feeding (e.g. aphids, mites) and chewing (caterpillars, beetles) herbivores as well as for beneficial insect groups, including parasitic Hymenoptera (Kaplan, 2012; Rodriguez-Saona et al., 2011).

When looking at the statistical correlations between the mean abundance of larvae of *M. aeneus* and *C. obstrictus* in the field experiment and laboratory results on volatile compounds of *B. napus* buds and flowers, several compounds in addition to acetic acid and methylbenzoate were correlated (Table 3). A positive

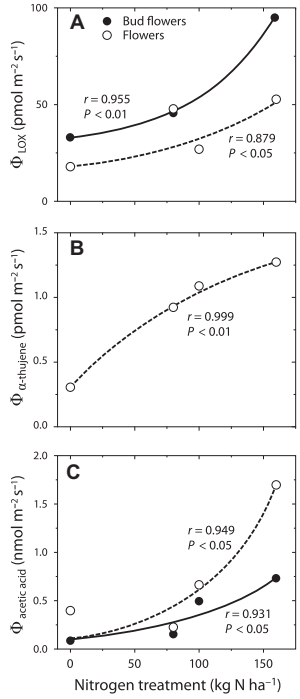

Fig. 2. The correlation between the emission of lipoxygenate pathway products (A), α-tujene (B) and acetic acid (C) in *Brassica napus* plants of at bud (●, solid lines) and flower (○, dash lines) stages, and N fertilization levels. Data were fitted by non-linear regressions in the form: $y = a \cdot e^{b \cdot x}$. No significant emission of α-tujene was detected in the bud stage.

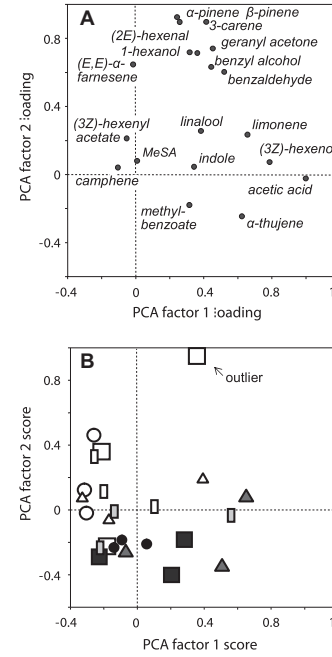

Fig. 3. Loading-plot (A) and score-plot (B) of PCA analysis based on the emission rates of VOCs (Table 2) of flowers and buds in *Brassica napus* at four different N fertilization treatments. In the loading plot, the numbers represent different VOCs of *Brassica* plants, while in the score plot, each symbol represents an individual *Brassica* plant in bud (empty symbols) or in flowering stage (filled symbols). Circles – N0; vertically extended rectangles – N80; squares – N100; triangles – N160. In the loading plot, the impact of chemical compounds increases with the distance from the origin of the co-ordinate system. Compounds that appear close to each other are co-varying.

correlation with mean *M. aeneus* larval number in flowers was detected for (3*Z*)-hexenyl acetate, camphene, 3-carene, limonene and indole emissions, and there were significant negative correlations with *β*-pinene, linalool, (*E*,*E*)-*α*-farnesene, methylbenzoate and benzaldehyde emissions. Depending on the direction of the correlation, these compounds could therefore be suggested as attractants or repellents for female pollen beetles.

Female *C. obstrictus* adults lay eggs into small developing siliques. They migrate to *B. napus* fields during the end of plants' flowering stage and are attracted to both flower and leaf extract odour (Evans and Allen-Williams, 1992). In our study, several LOX compounds and monoterpenes as well as methyl salicylate and acetic acid were emitted from flowers in higher concentrations with increasing N levels. Among these compounds with enhanced emissions in our study, (3*Z*)-hexenol and methyl salicylate have been shown to attract weevils (Bartlet et al., 1993). Unfortunately, only *α*-pinene, camphene and *β*-pinene showed a significant, although weak correlation to insect oviposition activity in our study (Table 3). Although *C. obstrictus* life cycle is better synchronized with *B. napus* winter varieties than *M. aeneus* (Alford et al., 2003; Veromann et al., 2006a,b; Williams, 2010), the abundance of weevils in the field was very low during both study years. This could also be a reason for small or no differences detected in the abundance of this pest at different N treatments. The highest average percentage of infected siliques was only 14.3% for the two study years, which is clearly lower than the threshold of 26% that has shown to potentially cause significant yield losses (Free and Williams, 1978; Lerin, 1984). Therefore additional studies on years with more pests and possibly also more VOC collections may provide more information about this insect – host plant communication system.

The main searching cue in the spring for *B. napus* pests' parasitoids is probably the odour of the crop (Williams and Cook, 2010). Jönsson et al. (2005) showed that the larval endoparasitoids of *M. aeneus* were attracted to yellow colour and odour from *B. napus* buds. However, parasitoids' host search has generally two phases: localization of hosts' habitat (plants) via plant volatiles from distance and thereafter localization of potential hosts (insects)

Table 3
Correlations between *M. aeneus* larval number and emission rates of volatiles from buds (bud stage), and *C. obstrictus* damaged pods and emission rates of volatiles from flowers (flower stage) in 2008–2009.

Volatile organic compound	*Meligethes aeneus*						*Ceutorhynchus obstrictus*					
	2008		2009		Mean		2008		2009		Mean	
	r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*
(3*Z*)-hexenyl acetate	0.19	ns	0.50	<0.001	0.37	<0.001	0.06	ns	0.14	ns	0.2	ns
α-pinene	−0.23	ns	−0.04	ns	−0.1	ns	0.11	ns	0.27	<0.05	0.2	<0.05
camphene	0.19	ns	0.50	<0.001	0.37	<0.001	0.11	ns	0.29	<0.05	0.2	<0.05
β-pinene	−0.38	<0.01	-0.44	<0.001	−0.39	<0.001	0.11	ns	0.27	<0.05	0.2	<0.05
3-carene	−0.05	ns	0.35	<0.01	0.20	<0.05	0.08	ns	0.19	ns	0.15	ns
Limonene	0.12	ns	0.52	<0.001	0.36	<0.001	0.03	ns	0.06	ns	0.06	ns
Linalool	−0.36	0.005	−0.67	<0.001	−0.53	<0.001	−0.08	ns	−0.23	ns	-0.16	ns
(*E*,*E*)-α-farnesene	−0.38	<0.01	−0.44	<0.001	−0.39	<0.001	−0.08	ns	−0.19	ns	−0.15	ns
Benzaldehyde	−0.34	<0.01	−0.45	<0.001	−0.39	<0.001	0.03	ns	0.06	ns	0.06	ns
Indole	0.12	ns	0.32	<0.05	0.24	<0.01	−0.05	ns	−0.15	ns	−0.1	ns
Methylbenzoate	−0.32	<0.05	−0.68	<0.001	−0.53	<0.001	0.05	ns	0.13	ns	0.09	ns
Acetic acid	0.19	ns	0.50	<0.001	0.37	<0.001	0.03	ns	0.06	ns	0.06	ns

Spearman correlation coefficient (r_s) and its statistical significance (*P*) were calculated for nitrogen treatments N0, N80, N100 and N160 pooled.

within the habitat (Godfray, 1994). In our study, the parasitoids' host searching success for *M. aeneus* differed between years, but on average, it was the greatest in the control plots with the fewest number of buds and also with the smallest number of available insect hosts. Based on these results, we can speculate that even at close proximity, the host search might be affected by cues, both from plants and the insects. Among VOCs in bud stage, methylbenzoate was detected at significantly greater levels in untreated plants and might therefore serve as a habitat attractant to pollen beetle parasitoids.

The parasitization of *C. obstrictus* larvae was high at all treatments and strongly correlated with host availability. Although there is very little information about *C. obstrictus* parasitoids' preferences, it is known that the main host searching cue for *T. perfectus* is the odour of frass from the third instar of *C. obstrictus* (Dmoch and Rutkowska-Ostrowska, 1978; Dmoch, 1998; Williams, 2003). This is in concordance with our results as nitrogen treatment did not seem to have any impact on parasitoid abundance when separated from the impact of the host larvae. The dose and/or chemical composition of volatiles are essential in plant–herbivore–carnivore communication. For example, 1-hexanol has been shown to be either attractant or repellent to the insects, depending on its release rate (Smart and Blight, 1997). In addition, indole, (3*Z*)-hexenol, (3*Z*)-hexenyl acetate, methyl salicylate has been found to attract certain parasitoids, including parasitoids of cruciferous pests (James, 2005; James and Grasswitz, 2005; Kaplan, 2012; Lee, 2010; Williams et al., 2008). The interpretation of the impact of N on this interaction is adding even more complexity because N availability affects both the direct and indirect defence system of plants (Chen et al., 2010). This was probably a reason for the lack of clear impact of nitrogen fertilization on most of the volatile compounds detected from oilseed *B. napus* plants in our study (Table 2), although the emissions often correlated with the insect damage assessments (Table 3). It is probable that in field conditions, plants under different nitrogen treatments could emit various bouquets of volatile compounds which in combination with visual cues might have been either attractive or repellent for overwintered adults of *M. aeneus* and *C. obstrictus*. Plants may also influence parasitoids' searching success indirectly via production of secondary defence compounds in response to pest damage or other biotic or abiotic stresses. As plant chemical defences can reduce the quality of the plant to the herbivore, it may be a signal of low host quality for the parasitoid (Godfray, 1994). Such combinations, however, are clearly very challenging to measure and interpret and need more attention in the future to help to understand these multi-level relationships in field conditions (Dicke and Baldwin, 2010; Kaplan, 2012; Mumm et al., 2008; Orre et al., 2010).

In the case of many other fungal diseases (e.g. Söchting and Verreet, 2004), nitrogen fertilization can enhance fungal damage. However, in our study nitrogen fertilization significantly decreased the average *A. brassicae* occurrence on winter *B. napus*. Since the initial infection with *A. brassicae* occurred probably in the flowering stage, we compared the disease scores with VOCs from flowering plants. Several monoterpenes, green leaf volatiles and acetic acid were produced at higher levels at increased N dosages (Table 2). Since there is very few information about the impact of these compounds to fungal infections of plants, additional experiments are needed to determine if all of these can have antifungal activities. However, acetic acid has been experimentally shown to inhibit fungal growth (Delaquis et al., 1999; Kang et al., 2003) and therefore we suggest that increased acetic acid emission was one of the reasons for lower disease abundance of the fungal pathogen at greater nitrogen applications.

Confirming several previous studies (e.g. Cook et al., 2007b), our results show a clear difference in VOCs and their concentrations between the bud and flower stage of *B. napus*. Among the 19 detected compounds, there were only two – methyl salicylate and acetic acid – that were produced at increased levels at greater N levels both at bud and flowering stages. The effect of N nutrition on other compounds might have either been absent or detectable only in combination with other factors. It is clear that the bouquet of the volatiles is changing with plant development, but it is also clear that the effects of the surrounding ecosystem should be considered. For instance, it is possible, that after the first insects feed on the *B. napus*, the plants may become more or less attractive for other individuals depending on the nutrient availability in the soil (Olson et al., 2009). Additionally, infections with pathogenic or endophytic micro-organisms may influence the plant emissions. Since the emission of volatiles is previously shown to be highly variable under different biotic and abiotic stresses (Copolovici et al., 2011; Holopainen and Gershenzon, 2010; Niinemets, 2010; Toome et al., 2010), additional studies with insects feeding on the plants are needed to continue the studies of the effect of N on *B. napus* pests.

The results of this study demonstrate that when optimizing the N fertilization of *B. napus* plants, the changes in plants and their effect to other organisms in the cropping system should also be considered. Natural populations of parasitoids could rather efficiently control the pest populations and if favourable conditions are created, it would enable to cut back on the pest control cost. Last, but not least, optimized use of all chemicals in *B. napus* cropping

systems would greatly decrease the environmental footprint of the production of this crop and therefore contribute to more environmentally friendly crop production and plant protection.

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Wold, S., Esbensen, K., Geladi, P., 1987. Principal component analysis. Chemometr. Intell. Lab. Syst. 2, 37–52.

CURRICULUM VITAE

First name: Riina
Last name: Kaasik
Date of Birth: September 10th 1984
Institution: Institute of Agricultural and Environmental Sciences Estonian University of Life Sciences, Kreutzwaldi 1, Tartu 51014, Estonia
Position: Specialist

Education:

2009–2014	PhD studies in, Estonian University of Life Sciences
2006–2009	MSc studies in Horticulture, Estonian University of Life Sciences
2003–2006	BSc studies in Horticulture, Estonian Agricultural University
2002–2003	Tartu Forseliuse High School
1991–2002	Nõo Secondary Science Gymnasium

Academic degree:

2009	Master's Degree, Thesis: Supercooling ability and cold hardiness in the horseradish flea beetle *Phyllotreta armoracia* (Koch) (Coleoptera: Chrysomelidae: Alticinae) Estonian Agricultural University

Foreign languages:

English – very good; German – lower intermediate, Russian – basic

Professional employment:

Since 2009	Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, specialist

Research interests:

Insect pests and their parasitoids of cruciferous crops, biocontrol.

Participation in research projects:

2013–2017	European Union 7th Framework Programme project No. 8-2/T12156PKTK: "Quantification of ecological services for sustainable agriculture", researcher.
2011–2014	Estonian Science Foundation Grant No 8895: "Impact of host plants on the major pests of cruciferous plants and their parasitoids in different cropping systems", PhD student/principal investigator/research stuff.
2010–2014	Target financed project No. SF 0170057s09: "Plant protection for sustainable crop production", PhD student/research stuff.
2009–2009	Estonian Science Foundation Grant No 7130:" The effects of food plants and microsporidiosis (Microsporidia, Nosematidae) on development and over-wintering physiology of insect pests on vegetable crops", PhD student/principal investigator/research stuff.
2008–2009	Estonian Science Foundation Grant No Delayed effects of sublethal doses of natural insecticides on pest and beneficial insects", PhD student/principal investigator/research stuff.

Awards and grants:

2014	Activity 8 of the ESF DoRa Programme, Archimedes foundation for participation in conference
2013	Activity 8 of the ESF DoRa Programme, Archimedes foundation for participation in conference
2013	IOBC meeting Student prize for research and presentation
2013	Activity 8 of the ESF DoRa Programme, Archimedes foundation for participation in conference
2012	Estonian Students Fund in USA, INC Scholarship
2012	Activity 8 of the ESF DoRa Programme, Archimedes foundation for participation in conference
2012	Doctoral School of Earth Sciences and Ecology Mobility support grant for participation in congress
2012	SA Archimedes DoRa 8 stipendium for self-education in laboratory
2011	SA Archimedes DoRa 8 stipendium for self-education in laboratory
2011	Doctoral School of Earth Sciences and Ecology Mobility support grant for PhD course

2011	Activity 8 of the ESF DoRa Programme, Archimedes foundation for participation in conference
2011	Estonian World Council, inc. Margot M and Herbert R Linna scholarship 2011/2012
2011	Estonian National Culture Fondation Zonta fund

Professional training:

2012	Animal ethics – from laboratory to wilderness, EULS, Estonia
2012	Chemical ecology 2, University of Tartu, Estonia
2012	Workshop-seminar: how to give a popular science talk I, University of Tartu, Estonia
2012	Study of oilseed rape pests and their parasitoids, Rothamsted Research, UK
2011	Statistical data analysis, University of Tartu, Estonia
2011	Practice learning in university teaching, EULS, Estonia
2011	Russian for beginners, EULS, Estonia
2011	Chemical ecology, University of Tartu, Estonia
2011	BeeNOVA PhD course Communication in Insects, University of Helsinki, Finland
2011	English for Specific Purposes: Practical conference communication, EULS, Estonia
2011	Study of oilseed rape pests and their parasitoids, Rothamsted Research, UK
2010	Academic writing and presentation, EULS, Estonia
2010	Mathematical statistics and modelling, EULS, Estonia
2010	Methodology of research, EULS, Estonia
2009	BeeNOVA PhD course Climate change Impacts on Pollinators, University of Helsinki, Finland
2009	Methods and analytical techniques to measure Biogenic Volatile Organic Compound (BVOC) emissions, EULS, Estonia
2009	Copyright and legal protection of intellectual property, EULS, Estonia
2009	Pedagogy of higher education, EULS, Estonia
2009	Philosophy of science, EULS, Estonia

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Amet: Spetsialist

Haridus:

2009–2014	Doktoriõpe entomoloogia erialal, Eesti Maaülikool
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2003–2006	Bakalaureuseõpe aianduse erialal, Eesti Põllumajandusülikool
2002–2003	Tartu Forseliuse Gümnaasium
1991–2002	Nõo Reaalgümnaasium

Teaduskraad:

2009	Magister, väitekiri: Mädarõika maakirbu *Phyllotreta armoracia* Koch (Crysomelida, Phyllotreta) allajahtumisvõimet ja külmataluvust mõjutavad faktorid, Eesti Maaülikool

Võõrkeelte oskus:

Inglise keel – väga hea; saksa keel – kesktase, vene keel – algtase

Teenistuskäik:

Alates 2009	Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, spetsialist

Teadusorganisatsiooniline tegevus:

Alates 2009	Eesti Taimekaitse Seltsi liige

Uurimustöö põhisuunad:

Ristõieliste kultuuride kahjurid ja nende parasitoidid, bioloogiline tõrje.

Osalemine uurimisprojektides:

2013–2017 Euroopa Liidu 7. raamprogrammi projekt 8-2/T12156PKTK „Ökosüsteemi teenuste roll jätkusuutlikus põllumajanduses“, põhitäitja.

2011–2014 Eesti teadusfondi grant 8895: “Peremeestaimede mõju ristõieliste õlikultuuride võtmekahjuritele ja nende parasitoididele erinevates viljelustingimustes”, põhitäitja.

2010–2014 Sihtfinantseeritav teema SF 0170057s09 “Taimekaitse jätkusuutlikule taimekasvatusele”, täitja.

2009–2009 Eesti teadusfondi grant 7130: “Toidutaimede ja mikrosporidioosi (Microsporidia, Nosematidae) mõju köögiviljakultuuride putukkahjurite arengule ja talvitusfüsioloogiale”, põhitäitja.

2008–2009 Eesti teadusfondi grant 6722: “Looduslike insektitsiidide subletaalsete dooside järeltoime kahjuritele ja kasuritele”, põhitäitja.

Teaduspreemiad ja stipendiumid:

2014 SA Archimedese DoRa 8 stipendium konverentsil osalemiseks

2013 SA Archimedese DoRa 8 stipendium konverentsil osalemiseks

2013 IOBC kohtumise tudengipreemia teadustöö ja ettekande eest

2013 SA Archimedese DoRa 8 stipendium konverentsil osalemiseks

2012 Eesti Üliõpilaste Toetusfond USAs stipendium 2012/2012

2012 SA Archimedese DoRa 8 stipendium konverentsil osalemiseks

2012 Maateaduste ja ökoloogia doktorikooli välissõidu stipendium kongressil osalemiseks

2012 SA Archimedese DoRa 8 stipendium enesetäiendamiseks välislaboris

2011 SA Archimedese DoRa 8 stipendium enesetäiendamiseks välislaboris

2011 Maateaduste ja ökoloogia doktorikooli välissõidu stipendium PhD kursusest osavõtuks

2011 SA Archimedese DoRa 8 stipendium konverentsil osalemiseks

2011 ÜEKN-i Margot ja Herbert Linna nimeline stipendium 2011/2012
2011 ERKF Zonta fondi stipendium 2011/2012

Erialane täiendamine:

2012 Loomaeetika, EMÜ, Eesti
2012 Keemiline ökoloogia 2, TÜ, Eesti
2012 Loodusteaduste doktorantide esinemisoskuste koolitusseminar I, TÜ, Eesti
2012 Täiendõpe teaduslaboris rapsi kahjurite ja nende parasitoidide alal, Rothamsted'i Uurimisinstituut, Suurbritannia
2011 Katseandmete analüüs, TÜ, Eesti
2011 Ülikooli pedagoogiline praktika, EMÜ, Eesti
2011 Vene keel algajatele, EMÜ, Eesti
2011 Keemiline ökoloogia, TÜ, Eesti
2011 BeeNOVA kraadiõppurite kursus doktorantidele: "Putukate kommunikeerumine", Helsingi Ülikool, Soome
2011 Inglise erialakeel. Konverentsisuhtlus: esitlus ja väitlus, EMÜ, Eesti
2011 Täiendõpe teaduslaboris rapsi kahjurite ja nende parasitoidide alal, Rothamsted'i Uurimisinstituut, Suurbritannia
2010 Akadeemiline kirjutamine ja esitlus, EMÜ, Eesti
2010 Matemaatiline statistika ja modelleerimine, EMÜ, Eesti
2010 Teadustöö metodoloogia, EMÜ, Eesti
2009 BeeNOVA kraadiõppurite kursus doktorantidele: "Kliimamuutuste mõju tolmeldajatele", Helsingi Ülikool, Soome
2009 Biogeensete lenduvate ühendite mõõtmismeetodid, EMÜ, Eesti
2009 Intellektuaalomandi kaitse ja autoriõigus, EMÜ, Eesti
2009 Kõrgkoolipedagoogika, EMÜ, Eesti
2009 Teadusfilosoofia, EMÜ, Eesti

LIST OF PUBLICATIONS

1.1. Publications indexed in the ISI Web of Science database:

Kaasik, **R**., Kovacs, G., Kaart, T., Metspalu, L., Williams, I.H., Veromann, E. 2014. *Meligethes aeneus* oviposition preferences, larval parasitism rate and species composition of parasitoids on *Brassica nigra*, *Raphanus sativus* and *Eruca sativa* compared with on *Brassica napus*. Biological control, 69, 65–71.

Kovacs, G., **Kaasik**, **R**., Metspalu, L., Williams, Ingrid H., Luik, Anne, Veromann, E. 2014. Could *Brassica rapa*, *Brassica juncea* and *Sinapis alba* facilitate the control of the cabbage seed weevil in oilseed rape crops? Biological control, 65(1), 124–129.

Veromann, E., Toome, M., Kännaste, A., **Kaasik**, **R**., Copolovici, L., Flink, J., Kovács, G., Narits, L., Luik, A., Niinemets, Ü. 2013. Effects of nitrogen fertilisation on insect pests, their parasitoids, plant diseases and volatile organic compounds in *Brassica napus*. Crop Protection, 43, 79–88.

Kaasik, **R**., Kovacs, G., Toome, M., Metspalu, L., Veromann, E. 2014. The relative attractiveness of *Brassica napus*, *B. rapa*, *B. juncea and Sinapis alba* to pollen beetles. BioControl 59(1), 19–28.

Hiiesaar, K., **Kaasik**, **R**., Williams, I.H., Svilponis, E., Jõgar, K., Metspalu, L., Mänd, M., Ploomi, A., Luik, A. 2012. Cold hardiness of horseradish flea beetle (*Phyllotreta armoraciae* (Koch)). Zemdirbyste=Agriculture, 99(2), 203–208.

Veromann, E., Metspalu, L., Williams, I.H., Hiiesaar, K., Mand, M., **Kaasik**, **R**., Kovács, G., Jogar, K., Svilponis, E., Kivimagi, I., Ploomi, A., Luik, A. 2012. Relative attractiveness of *Brassica napus*, *Brassica nigra*, *Eruca sativa* and *Raphanus sativus* for pollen beetle (*Meligethes aeneus*) and their potential for use in trap cropping. Arthropod-Plant Interactions, 6(3), 385–394.

Veromann, E., Williams, I.H., **Kaasik**, **R**., Luik, A. 2011. Potential of parasitoids to control populations of the weevil *Ceutorhynchus obstrictus* (Marsham) on winter oilseed rape. International Journal of Pest Management, 57(1), 85–92.

3.4. Papers published in the proceedings of international conferences:

Kaasik, **R**., Watts, N.P., Murray, D.A., Veromann, E., Cook, S.M. 2013. Effects of monitoring position and time of day on pollen beetle numbers in crops of oilseed rape. Bulletin IOBC/wprs, Integrated Control in Oilseed Crops, 96, 123–131.

Kovács, G., **Kaasik**, **R**., Metspalu, L., Veromann, E. 2013. The attractiveness of wild cruciferous plants on the key parasitoids of *Meligethes aeneus*. Bulletin IOBC/wprs, Integrated Control in Oilseed Crops, 96, 81–92.

Kaasik, **R**., Kovács, G., Pehme, S., Veromann, E. 2013. The effect of companion planting on the abundance of pest complex and its parasitism rate on white cabbage. NJF Seminar 461 "Organic farming as a Driver for Change": 21–23.08.2013 Bredsten, Denmark. NJF Report 9(3): 175–176.

Kovács, G., **Kaasik**, **R**., Veromann, E. 2013. The impact of companion planting on the abundance of Lepidopteran pests on white cabbage. Future IPM in Europe, 19–21.03.2013, Riva del Garda, Italy. (on USB stick)

Kaasik, **R**., Kovács, G., Luik, A., Veromann, E. 2012. The impact of companion planting on the parasitism rate of the small white butterfly *Pieris rapae* (Lepidoptera: Pieridae). Bulletin IOBC/wprs, Landscape Management For Functional Biodiversity, 75, 109–113.

3.5. Papers published in the proceedings of Estonian conferences:

Kaasik, **R**., Kovács, G., Veromann, E. 2013. Kahjurite ja parasitoidide talvitumine rapsipõllul ja selle serva-aladel. Agronoomia 2013, 144–149.

Kaasik, **R**., Kovács, G., Veromann, E. 2012. Kahjurite arvukuse hindamise tähtsus suurkapsaliblika näitel. Agronoomia 2012, 129–134.

Kovacs, G., **Kaasik**, **R**., Luik, A., Veromann, E. 2012. Kivikilbik meelitab kapsakoid. Mahepõllumajanduse arengusuunad – teadlaselt mahepõllumajandusele, 50–52.

Kovács, G., **Kaasik**, **R**., Veromann, E. 2012. Kõdrasääsk (*Dasineura brassicae*) – uus kahjustaja rapsil. Agronoomia 2012, 141–144.

Kaasik, **R**., Kovács, G., Luik, A., Veromann, E. 2012. Seltsilistaimed kapsaliblikate mõjutajatena. Mahepõllumajanduse arengusuunad – teadlaselt mahepõllumajandusele, 38–40.

Veromann, E., **Kaasik**, **R**., Kovács, G., Luik, A. 2012. Till aitab kapsast öölase eest peita. Mahepõllumajanduse arengusuunad – teadlaselt mahepõllumajandusele, 92–94.

Kaasik, **R**., Kovács, G., Luik, A., Veromann, E. 2011. Seltsilistaimede mõju entomofauna mitmekesisusele valgel peakapsal. Agronoomia, 165–172.

VIIS VIIMAST KAITSMIST

MEELIS SEEDRE

DISTURBANCE EFFECTS ON BOREAL FOREST ECOSYSTEM CARBON DYNAMICS

HÄIRINGUREŽIIMI MÕJU BOREAALSE METSAÖKOSÜSTEEMI SÜSINIKUVOOGUDELE

Professor **Kalev Jõgiste**, professor **Han Chen** (*Lakehead University, Canada*)

16. detsember 2013

HEDI HARZIA

ASSOCIATIONS BETWEEN METABOLIC PROFILE AND COAGULATION ABILITY OF BOVINE MILK, EFFECT OF FEEDING AND LACTATION STAGE

LEHMAPIIMA METABOOLSE PROFIILI JA LAAPUMISE VAHELISED SEOSED, SÖÖTMISE JA LAKTATSIOONIPERIOODI MÕJU

Professor *emeritus* **Olav Kärt**, professor **Ursel Soomets** (Tartu Ülikool), vanemteadur **Kalle Kilk** (Tartu Ülikool)

20. detsember 2013

PIRET KALMUS

CLINICAL MASTITIS IN ESTONIA: DIAGNOSIS, TREATMENT EFFICACY AND ANTIMICROBIAL RESISTANCE OF PATHOGENS IN ESTONIA

KLIINILISTE MASTIITIDE DIAGNOOSIMINE, RAVI TULEMUSLIKKUS JA PATOGEENIDE ANTIMIKROOBNE RESISTENTSUS EESTIS

Professor **Toomas Orro**, dotsent **Kalle Kask**

23. jaanuar 2014

OLGA TAMMEORG

EFFECTS OF NATURAL AND ANTHROPOGENIC PRESSURES ON VARIATIONS IN THE WATER QUALITY OF LARGE LAKES

LOODUSLIKE JA INIMTEKKELISTE SURVETEGURITE TOIME VEE KVALITEEDI MUUTLIKKUSELE SUURJÄRVEDES

Vanemteadur **Tõnu Möls**, vanemteadur **Külli Kangur**

3. aprill 2014

ARE KAASIK

THE DETECTION OF LAND USE CHANGE AND ITS INTERACTIONS WITH BIOTA IN ESTONIAN RURAL LANDSCAPES

MAAKASUTUSMUUTUSTE MÕJU MAAPIIRKONDADE MAASTIKULISELE JA BIOLOOGILISELE MITMEKESISUSELE

Professor **Kalev Sepp**

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